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Evaluating photosynthetic models and their potency in assessing plant responses to changing oxygen concentrations: a comparative analysis of A_n-C_a and A_n-C_i curves in *Lolium perenne* and *Triticum aestivum*

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Accurate determination of photosynthetic parameters is essential for understanding how plants respond to environmental changes. In this study, we evaluated the performance of the Farquhar-von Caemmerer-Berry (FvCB) model and introduced a novel model to fit photosynthetic rates against ambient CO₂ concentration (A_n-C_a) and intercellular CO₂ concentration (A_n-C_i) curves for *Lolium perenne* and *Triticum aestivum* under 2% and 21% O₂ conditions. We observed significant discrepancies in the FvCB model's fitting capacity for A_n-C_a and A_n-C_i curves across different oxygen regimes, particularly in estimates of key parameters such as the maximum carboxylation rate (V_{cmax}), the day respiratory rate (R_{day}), and the maximum electron transport rate for carbon assimilation (J_{A-max}). Notably, under 2% and 21% O₂ conditions, the values of V_{cmax} and R_{day} derived from A_n-C_a curves using the FvCB model were 46.98%, 44.37%, 46.63%, and 37.66% lower than those from A_n-C_i curves for *L. perenne*, and 47.10%, 44.30%, 47.03%, and 37.36% lower for *T. aestivum*, respectively. These results highlight that the FvCB model yields significantly different V_{cmax} and R_{day} values when fitting A_n-C_a versus A_n-C_i curves for these two C₃ plants. In contrast, the novel model demonstrated superior fitting capabilities for both A_n-C_a and A_n-C_i curves under 2% and 21% O₂ conditions, achieving high determination coefficients ($R^2 \geq 0.989$). Key parameters such as the maximum net photosynthetic rate (A_{max}) and the CO₂ compensation point (I) in the presence of R_{day} showed no significant differences across oxygen concentrations. However, the apparent photorespiratory rate (R_{pa0}) and photorespiratory rate (R_{p0}) derived from A_n-C_i curves consistently exceeded those from A_n-C_a curves for both plant species. Furthermore, R_{pa0} values derived from A_n-C_a curves closely matched observed values, suggesting that A_n-C_a curves more accurately

reflect the physiological state of plants, particularly for estimating photorespiratory rates. This study underscores the importance of selecting appropriate CO₂-response curves to investigate plant photosynthesis and photorespiration under diverse environmental conditions, thereby ensuring a more accurate understanding of plant responses to changing environments

KEYWORDS

photosynthesis models, C₃ plants, FvCB model, parameter estimation, CO₂-response to photosynthesis, apparent photorespiratory rate

1 Introduction

In the context of ongoing global climate change and the persistent increase in atmospheric CO₂ concentrations, studying the response models of plant photosynthesis to intercellular CO₂ concentration (C_i) (see [Supplementary Table S1](#) for the list of abbreviations) and ambient CO₂ concentration (C_a) is of critical importance. Photosynthesis, the process by which plants convert CO₂ into organic matter using light energy, plays a crucial role in the carbon cycle and the energy flow within ecosystems ([Sergio et al., 2015](#); [Kolari et al., 2014](#); [Roberta et al., 2024](#)). This process is influenced by both light intensity and CO₂ concentrations, making the development of accurate photosynthesis models essential for predicting plant growth and ecosystem changes ([Eric et al., 2019](#); [Pleban et al., 2020](#); [Taylor et al., 2024](#)).

The response models of photosynthesis to C_i (A_n - C_i model, where A_n represents the net photosynthetic rate) and to C_a (A_n - C_a model) are fundamental for understanding plant photosynthesis, each addressing different aspects. The A_n - C_i model primarily describes the relationship between the internal CO₂ concentration within plant leaves and A_n . This model incorporates internal gas exchange and biochemical processes, such as the carboxylation reaction of Rubisco ([Kelly et al., 2016](#); [Ye et al., 2024](#)). C_i denotes the CO₂ concentration in the air space around mesophyll cells, which is influenced by stomatal conductance ([Miner and Bauerle, 2017](#); [Taylor et al., 2024](#)). This model is typically used to analyze photosynthetic performance under various environmental conditions, including changes in light, temperature, and CO₂ concentrations ([De Kauwe et al., 2016](#); [Yiotis et al., 2021](#); [Xiong et al., 2022](#)). Conversely, the A_n - C_a model focuses on the relationship between ambient CO₂ concentration and plant photosynthesis. This model evaluates the impact of changes in atmospheric CO₂ on photosynthesis, particularly in the context of global climate change and rising CO₂ levels ([Eric et al., 2019](#); [Qiu and Katul, 2020](#)). It helps predict future impacts on plant growth and ecosystem dynamics. In practical applications, the A_n - C_i model provides insights into the biochemical mechanisms of photosynthesis, while the A_n - C_a model is more frequently used in ecological and climate change research ([Kelly et al., 2016](#); [Miner and](#)

[Bauerle, 2017](#); [Xiong et al., 2022](#)). Both models are invaluable tools in plant physiology and global change biology.

As we explore the complex ways in which plant photosynthesis responds to variations in CO₂ levels, a plethora of models has been developed by scientists. These models can be broadly categorized into two types: empirical and biochemical. Among the empirical models, the Michaelis-Menten (M-M) model stands out due to its foundation in enzyme kinetics, while the exponential equation model provides a mathematical framework for describing the photosynthetic response ([Watling et al., 2000](#); [Sharkey et al., 2007](#); [Silva-Pérez et al., 2017](#)), offering a refined method for fitting photosynthetic curves. These empirical models are utilized to fit the A_n - C_a or A_n - C_i curves of plants, enabling the extraction of key parameters such as the maximum net photosynthetic rate (A_{max}), which indicates the upper limit of photosynthetic capacity, the CO₂ compensation point with day respiratory rate (Γ), which reveals the CO₂ level at which photosynthesis balances respiration, and the apparent photorespiratory rate (R_{pa0}) at CO₂ concentration approaching 0 $\mu\text{mol mol}^{-1}$, a measure of the energy invested in photorespiration ([Leuning, 1995](#); [Medlyn et al., 2011](#); [Morfopoulos et al., 2014](#)). These three pivotal photosynthetic parameters are quantifiable ([Medlyn et al., 2011](#); [Morfopoulos et al., 2014](#); [Burnett et al., 2019](#)). Consequently, regardless of the model used and whether it is for the A_n - C_a or A_n - C_i curve, the parameters derived from these models should closely match the observed data without significant discrepancies. It is only under these conditions that we can deem a model to be effective. Apparently, the M-M model and the exponential equation model, though both being asymptotic functions, can't always accurately depict net CO₂ assimilation rates reductions beyond the TPU-limitation phase in some plant species ([Watling et al., 2000](#); [Silva-Pérez et al., 2017](#)). Furthermore, these two models are unable to directly estimate the critical transition point from Ribulose-1,5-bisphosphate (RuBP)-limited to TPU-limited conditions (C_{TPU}).

Among the biochemical models, the one developed by [Farquhar et al. \(1980\)](#) and its subsequent modifications ([Harley and Sharkey, 1991](#)) are central. The model introduced by [Farazdaghi and Edwards \(1988\)](#) also contributes to the field. The Farquhar model, widely known as the FvCB model, has been extensively analyzed for

its biochemical mechanisms and is used to fit the A_n-C_i curve of C_3 plants (Dubois et al., 2007; Fan et al., 2011; Busch and Sage, 2017; Rogers et al., 2017; Walker et al., 2017; Yin et al., 2021). This model provides five crucial parameters: the maximum electron transport rate (J_{A-max}) for photosynthesis, the maximum carboxylation rate (V_{cmax}), triose phosphate utilization rate (V_{TPU}), day respiratory rate (R_{day}), CO_2 compensation point (Γ^*) in absence of R_{day} and mesophyll conductance (g_m) (Long and Bernacchi, 2003; Norby et al., 2017; Xiao et al., 2021). Despite its extensive use in studying the photosynthetic response of C_3 plants to environmental changes such as light, temperature, CO_2 concentration, and nitrogen (N) nutrition (Bellasio et al., 2015; Sharkey, 2016; Busch and Sage, 2017; Anderegg et al., 2018; Cheah and The, 2020; Han et al., 2020; Yin et al., 2021; Yin and Amthor, 2024), the FvCB model has limitations. It cannot directly estimate parameters like A_{max} , Γ , and R_{pa0} , as well as is specific to C_3 plants.

In the current research field, the A_n-C_i curves of plants are typically analyzed using biochemical models to derive key photosynthetic parameters (Farquhar et al., 1980; Harley and Sharkey, 1991; Fan et al., 2011; Vijayakumar et al., 2024). However, research on fitting the A_n-C_a curve is limited, probably because C_i is directly involved in photosynthetic carboxylation while ambient C_a only indirectly affects C_i (Long and Bernacchi, 2003). Moreover, there is a paucity of studies examining whether significant differences exist between important photosynthetic parameters (e.g., A_{max} , Γ , and R_{pa0}) derived from the A_n-C_a and A_n-C_i curves and the observed data (Busch and Sage, 2017). Similarly, the consistency of parameters such as J_{A-max} , V_{cmax} , Γ^* , V_{TPU} , and R_{day} obtained from fitting gas exchange data (i.e., A_n-C_a and A_n-C_i curves) with biochemical models, remains underexplored (Medlyn et al., 2011; Morfopoulos et al., 2014; Yin et al., 2021; Smith et al., 2023). Accurately determining these parameters is essential for understanding plant responses to ambient CO_2 variations, evaluating carbon assimilation efficiency, and assessing adaptability to climate change. Thus, a comparative analysis of model predictions and measured data is particularly critical.

In this study, we begin with a clear and concise explanation of the FvCB model, initially introduced by Farquhar et al. in 1980. Subsequently, we introduce a new model. This model describes the CO_2 -response curve of photosynthesis and incorporates an explicit term for R_{day} . Additionally, we also introduce another version of the model that does not explicitly define R_{day} . Then, we showcase how to apply both the FvCB model and our newly developed model to fit A_n-C_a and A_n-C_i curves, respectively, in order to estimate key photosynthetic and biochemical parameters. Finally, we utilize a modeling-observation intercomparison approach to assess the photosynthetic parameters derived from A_n-C_a and A_n-C_i curves for both the FvCB model and our newly developed model. For the FvCB model, these parameters include V_{cmax} , J_{A-max} , Γ^* , V_{TPU} , and R_{day} . For the new model, they encompass A_{max} , C_{TPU} , Γ , CO_2 compensation point (Γ^*) in the absence of R_{day} and R_{pa0} . We then determine which parameters exhibit different values when derived from the A_n-C_a and A_n-C_i curves. Ultimately, we evaluate whether there are significant differences in the estimated parameters obtained from these two types of curves. Through this

methodological exploration, we aim to provide novel insights and tools to enhance research in plant photosynthesis.

2 Materials and methods

2.1 FvCB model description

The FvCB model considers that the carbon assimilation process of C_3 plants includes: the Rubisco enzyme activity limitation stage, the RuBP regeneration limitation stage, and the triose phosphate utilization (V_{TPU}) limitation stage.

In the Rubisco limitation stage (Farquhar et al., 1980; Dubois et al., 2007; Sharkey et al., 2007; Miao et al., 2009; Ellsworth et al., 2015; Yin et al., 2021), the following equation is used:

$$A_c = \frac{V_{c,max}(C_i - \Gamma^*)}{C_i + K_c(1 + O/K_o)} - R_{day} \quad (1)$$

where $V_{c,max}$ is the maximum velocity of the carboxylase ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$); C_i is the intercellular CO_2 concentration; K_c and K_o are the Michaelis-Menten constants for CO_2 and O_2 , respectively (Farquhar et al., 1980; Silva-Pérez et al., 2017); O is the partial pressure of oxygen at the site of Rubisco. R_{day} is the day respiratory rate.

During the RuBP-limited phase of photosynthesis, if the regeneration of RuBP is predominantly constrained by the availability of NADPH, then the following equation can be applied:

$$A_j = J \frac{C_i - \Gamma^*}{4C_i + 8\Gamma^*} - R_{day} \quad (2)$$

where A_j is the carbon assimilation rate limited by RuBP regeneration capacity. At light saturation, J is equal to J_{A-max} in Equation 2 (Farquhar et al., 1980; Gu et al., 2010; von Caemmerer, 2013; Farquhar and Busch, 2017; Yin et al., 2021).

When RuBP regeneration is co-determined by the availability of both NADPH and ATP, the equation assumes a slightly modified form:

$$A_j = J \frac{C_i - \Gamma^*}{4.5C_i + 10.5\Gamma^*} - R_{day} \quad (3)$$

At light saturation, J is equal to J_{A-max} in Equation 3 (von Caemmerer, 2000; Long and Bernacchi, 2003; Yin et al., 2004; Yin et al., 2009; Lenz et al., 2010; Bernacchi et al., 2013).

In the TPU limitation stage, we have:

$$A_p = 3V_{TPU} \frac{C_i - \Gamma^*}{C_i - (1 + 3\alpha_G)\Gamma^*} - R_{day} \quad (4)$$

where A_p is the carbon dioxide assimilation rate when the utilization of phosphate glucose is limited. α_G is the proportion of glycolic acid not returned to chloroplasts and is related to the release of phosphate (Dubois et al., 2007). α_G is between [0, 1]. While α_G is equal to 0, Equation 4 is expressed as $A_p = 3V_{TPU} - R_{day}$ (Long and Bernacchi, 2003; Bernacchi et al., 2013).

Based on the analysis above, it can be inferred that fitting the A_n-C_a and A_n-C_i curves separately using the FvCB model should

yield different values for V_{cmax} , $J_{\text{A-max}}$, and R_{day} , while the values for V_{TPU} and Γ_* should be the same. For V_{TPU} and Γ_* , it is not possible for their values to change simply because C_a or C_i is altered. This should be consistent with the actual situation.

On the other hand, experimental methodologies facilitate the accurate quantification of whole-chain electron transport, represented by J_f , through the application of fluorescence techniques. As elucidated by von Caemmerer (2000) and Long and Bernacchi (2003), J_f is partitioned among several pivotal processes, with photosynthesis being a principal recipient and denoted by J_A . Beyond photosynthesis, J_f contributes to other synthetic and electron-consuming pathways, including photorespiration (J_O), the reduction of nitrate to ammonium (J_{Nit}), and the light-driven oxygen uptake via the Mehler ascorbate peroxidase (MAP) reaction (J_{MAP}). Consequently, the relationship can be encapsulated as $J_f = J_A + J_O + J_{\text{Nit}} + J_{\text{MAP}}$. The analysis underscores a critical correlation between J_f and J_A : the magnitude of J_f must necessarily surpass that of J_A . This inference arises from the understanding that J_f encompasses not only the electrons associated with J_A . It also includes those in other synthetic and metabolic pathways, such as J_O , J_{Nit} , and J_{MAP} . Consequently, it can be deduced that the maximum rate of photosynthesis ($J_{\text{A-max}}$) as determined by the FvCB model must be inherently lower than the maximum rate of whole-chain electron transport ($J_{\text{f-max}}$).

In recent years, significant progress has been made in the field of plant photosynthesis models. Among them, the FvCB model has also achieved remarkable advancements in aspects such as dynamic light response expansion and multi-scale coupling models. Under dynamic light environments, stomatal conductance also changes dynamically and interacts with photosynthesis. Previous studies have coupled the dynamic stomatal model with the FvCB model (Zhang et al., 2017; Yin and Amthor, 2024). They found that by using an improved version of the Ball-Berry stomatal model, the dynamic effects of factors such as light intensity, carbon dioxide concentration, and air humidity on stomatal conductance can be considered. Stomatal conductance (g_s) can be expressed as: $g_s = g_0 + m \times A_n / C_a \times h_s$, where g_0 is the minimum stomatal conductance, m is an empirical coefficient, A_n is the net photosynthetic rate, C_a is the atmospheric CO_2 concentration, and h_s is the relative humidity of the air at the leaf surface. Under dynamic light environments, changes in light intensity affect the photosynthetic rate, which in turn dynamically adjusts the stomatal conductance through the stomatal model and then feedback-affects photosynthesis. This enables the model to more realistically simulate the photosynthesis-stomatal coupling process of plants under dynamic light environments.

2.2 A new model for describing the CO_2 -response curve of photosynthesis with an explicit R_{day}

In order to estimate key photosynthetic parameters such as A_{max} , C_{TPU} and Γ_* , we have developed a new model describing CO_2 -response curves of photosynthesis (A_n - C curves) (hereafter referred

to Model I). The new model can be written as follows:

$$A_n = \alpha_c \frac{1 - \beta_c C}{1 + \gamma_c C} (C - \Gamma_*) - R_{\text{day}} \quad (5)$$

where A_n is the net photosynthetic rate; Γ_* is the CO_2 compensation point in the absence of R_{day} . α_c , β_c and γ_c are three coefficients that depend on plant characteristics and environmental conditions, and they are independent of C . C can represent both ambient CO_2 concentration (C_a) and intercellular CO_2 concentration (C_i).

In addition, Equation 5 can be rearranged as:

$$A_n = \alpha_c \frac{1 - \beta_c C}{1 + \gamma_c C} C_i - \alpha_c \frac{1 - \beta_c C}{1 + \gamma_c C} \Gamma_* - R_{\text{day}} \quad (6)$$

In contrast to Equation 1, the initial term represents the gross rate of photosynthesis, while the subsequent term denotes the actual rate of photorespiration (R_p), assuming no contribution from R_{day} Equation 6. Consequently, R_p can be expressed as follows:

$$R_p = \alpha_c \frac{1 - \beta_c C}{1 + \gamma_c C} \Gamma_* \quad (7)$$

In Equation 7, R_p will decrease with C . Therefore, it can be used to investigate the relationship between R_p and C under different environmental factors. Specially, while $C = 0 \mu\text{mol mol}^{-1}$, $R_{p0} = \alpha_c \Gamma_*$. In this scenario, theoretically, R_{p0} will take on two different values when either C_a or C_i is at $0 \mu\text{mol mol}^{-1}$. In practice, there is only a single value that defines the CO_2 -response curve for photosynthesis. Consequently, the R_{p0} value serves as a benchmark to assess the plausibility of different response types. Specifically, the proximity of the R_{p0} values derived from fitting A_n - C_a and A_n - C_i curves to the actual measured data is one of the criteria used to evaluate which response type is more justifiable.

Supposing that R_{day} approximates a constant or is independent of C , the first derivative of Equation 5 is expressed as follows:

$$\frac{dA_n}{dC} = \alpha_c \frac{1 - 2\beta_c C - \beta_c \gamma_c C^2 + (\beta_c + \gamma_c)\Gamma_*}{(1 + \gamma_c C)^2} \quad (8)$$

where dA_n/dC is the slope of the A_n - C curve, and dA_n/dC decreases with increasing C . As C tends to zero in Equation 8, dA_n/dC equals $\alpha_c[1 + (\beta_c + \gamma_c)\Gamma_*]$, and it is referred to as the initial slope of the A_n - C curve ($\alpha_0 = \alpha_c[1 + (\beta_c + \gamma_c)\Gamma_*]$). When dA_n/dC equals zero, C_{TPU} can be calculated, then dA_n/dC will be negative when C surpasses C_{TPU} . Therefore, Equation 5 is a non-asymptotic function.

When $dA_n/dC = 0$, C_{TPU} can be calculated Equation 9:

$$C_{\text{TPU}} = \frac{\sqrt{(\beta_c + \gamma_c)(1 + \gamma_c \Gamma_*)/\beta_c} - 1}{\gamma_c} \quad (9)$$

And A_{max} can be calculated Equation 10:

$$A_{\text{max}} = \alpha_c \left[\frac{\sqrt{\beta_c + \gamma_c} - \sqrt{\beta_c(1 + \gamma_c \Gamma_*)}}{\gamma_c} \right]^2 - R_{\text{day}} \quad (10)$$

In addition, when A_n equals zero in Equation 5, Γ can be calculated Equation 11:

$$\Gamma = \frac{\left(1 + \beta_c \Gamma_* - \frac{\gamma_c R_{\text{day}}}{\alpha_c}\right) - \sqrt{\left(1 + \beta_c \Gamma_* - \frac{\gamma_c R_{\text{day}}}{\alpha_c}\right)^2 - 4\beta_c \left(\frac{R_{\text{day}}}{\alpha_c} + \Gamma_*\right)}}{2\beta_c} \quad (11)$$

Indeed, in practical terms, there is but a single set of values for A_{max} and Γ that characterizes the CO_2 -response curve of photosynthesis. These values are pivotal as they provide a reference point to evaluate the reasonableness of various response types. Notably, the closeness of the A_{max} and Γ values, as determined by fitting the A_n - C_a and A_n - C_i curves, to the observed data obtained serves as a critical criterion. This comparison helps in ascertaining which response type is more rational and aligned with the actual physiological processes of photosynthesis.

2.3 A new model for describing the CO_2 -response curve of photosynthesis without an explicit R_{day}

Since precisely measuring R_{day} in plants remains a challenging (Atkin and Tjoelker, 2003; Yin et al., 2009) (see Tcherkez et al., 2017 for a comprehensive review), we have developed an alternative model. It can not only accurately fit the CO_2 -response curve of photosynthesis (A_n - C curve) but also minimize potential fitting discrepancies due to different choices of R_{day} (hereafter referred to Model II). The model is expressed as follows:

$$A_n = \alpha_{c1} \frac{1 - \beta_{c1} C}{1 + \gamma_{c1} C} (C - \Gamma) \quad (12)$$

where α_{c1} , β_{c1} , and γ_{c1} are three coefficients that depend on plant characteristics and environmental conditions; Γ is the photorespiratory CO_2 compensation point in presence of R_{day} (Farquhar et al., 1980; Long and Bernacchi, 2003). Furthermore, considering the influence of R_{day} , it is anticipated that the coefficients of α_{c1} , β_{c1} , and γ_{c1} in Equation 12 will differ from those of α_c , β_c and γ_c in Equation 5.

In addition, Equation 12 can be rearranged as:

$$A_n = \alpha_{c1} \frac{1 - \beta_{c1} C}{1 + \gamma_{c1} C} C - \alpha_{c1} \frac{1 - \beta_{c1} C}{1 + \gamma_{c1} C} \Gamma \quad (13)$$

In Equation 13, the first term is the gross photosynthetic rate, and the second term is the apparent photorespiration rate (R_{pa}) including R_{day} . Therefore, R_{pa} can be expressed as:

$$R_{\text{pa}} = \alpha_{c1} \frac{1 - \beta_{c1} C}{1 + \gamma_{c1} C} \Gamma \quad (14)$$

In Equation 14, R_{pa} decreases as C increases. Therefore, it can be utilized to explore the relationship between R_p and C for all plant species under any environmental conditions. Indeed, when C is exactly $0 \mu\text{mol mol}^{-1}$, $R_{\text{pa}0}$ takes on a particular value. Theoretically, one might expect $R_{\text{pa}0}$ to have two different values corresponding to either C_a or C_i being at $0 \mu\text{mol mol}^{-1}$. However, in actuality, there is but a single value that delineates the CO_2 -response curve for photosynthesis. This singular C_a or C_i value thus becomes an essential criterion for assessing the plausibility of various response

types. Notably, the closeness of the $R_{\text{pa}0}$ values, derived from fitting A_n - C_a and A_n - C_i curves, to the observed data is a pivotal factor in judging which response type is more reasonable.

The first derivative of Equation 12 may be expressed as follows:

$$\frac{dA_n}{dC} = \alpha_{c1} \frac{1 - 2\beta_{c1} C - \beta_{c1} \gamma_{c1} C^2 + (\beta_{c1} + \gamma_{c1}) \Gamma}{(1 + \gamma_{c1} C)^2} \quad (15)$$

where dA_n/dC is the slope of the A_n - C curve, and dA_n/dC decreases with increasing C . As C tends to zero in Equation 15, dA_n/dC equals $\alpha_{c1} [1 + (\beta_{c1} + \gamma_{c1}) \Gamma_*]$, and it is referred to as the initial slope of the A_n - C curve (i.e., $\alpha_0 = \alpha_{c1} [1 + (\beta_{c1} + \gamma_{c1}) \Gamma_*]$). dA_n/dC equals zero when C equals to C_{TPU} , then dA_n/dC will be negative when C surpasses C_{TPU} . It is important to acknowledge that the new model, distinguishes between two distinct values for the A_n - C curve: one is $C_{i,\text{TPU}}$, which represents A_n - C_i , and the other is $C_{a,\text{TPU}}$, which corresponds to the A_n - C_a curve. This distinction is crucial for accurately modeling and understanding the photosynthetic responses in plants.

Therefore, while the $dA_n/dC = 0$, C_{TPU} is calculated by:

$$C_{\text{TPU}} = \frac{\sqrt{(\beta_{c1} + \gamma_{c1})(1 + \gamma_{c1} \Gamma)/\beta_{c1}} - 1}{\gamma_{c1}} \quad (16)$$

And A_{max} can be obtained as:

$$A_{\text{max}} = \alpha_{c1} \left[\frac{\sqrt{\beta_{c1} + \gamma_{c1}} - \sqrt{\beta_{c1}(1 + \gamma_{c1} \Gamma)}}{\gamma_{c1}} \right]^2 - R_{\text{day}} \quad (17)$$

Besides C_{TPU} and A_{max} can be calculated by Equations 16, 17, respectively, Γ can also be estimated by Equation 12. Indeed, in practical applications, there is but one definitive set of values for A_{max} and Γ that characterizes the CO_2 -response curve of photosynthesis. These values are crucial as they serve as a benchmark against which the reasonableness of different response types can be assessed. Importantly, the proximity of the A_{max} and Γ values, derived from fitting the A_n - C_a and A_n - C_i curves, to the actual observed data is a key criterion. This comparison is essential for determining which response type is more rational and in accordance with the true physiological mechanisms of photosynthesis.

Furthermore, it is important to acknowledge that Equations 5, 12 are fundamentally equivalent when it comes to determining photosynthetic parameters like A_{max} and Γ , irrespective of whether A_n - C_a curves or A_n - C_i curves are being fitted.

2.4 Plant materials

In this study, two typical C_3 plants, namely *Lolium perenne* L. (Zhongxin 830) and *Triticum aestivum* L. (Jimai 22), were selected as the experimental materials. As two important crops, their photosynthetic characteristics display the typical carbon assimilation process of C_3 plants. Their photosynthesis is highly sensitive to environmental conditions such as light intensity, carbon dioxide concentration, and water supply (Höglind et al., 2011; Pshenichnikova et al., 2019). Therefore, these two crops are

highly suitable for a comparative study of the differences between the FvCB model (applicable only to C_3 plants) and the new model in fitting the A_n-C_a and A_n-C_i curves and estimating key photosynthetic parameters under different oxygen concentration conditions. The two experimental materials were sown in mid-October 2022 and managed conventionally in the field. Data were collected on sunny days from April 28 to May 10, 2023. *T. aestivum* was at the booting stage to the initial flowering stage, with a plant height of 60–70 cm, and its flag leaf was selected for measurement. *L. perenne* was at the booting stage, with a plant height of about 1.3 m, and the first leaf below its flag leaf was selected for measurement.

2.5 Gas exchange data measurement

A portable photosynthesis system (LI-6400-40, LI-COR INC., USA) was used to collect data on sunny days from 9:00 to 11:30 and 14:00 to 17:00, with air temperatures at $30.3 \pm 2.5^\circ\text{C}$ and maximum midday light intensity of about $1,600 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. After a 1.5- to 2-hour induction under natural light, the device was turned on for preheating and checked. The oxygen concentration in the fluorescence leaf chamber is controlled by connecting external gas cylinders filled with different gas mixtures. The oxygen concentrations are set at 2% (2% oxygen and 98% nitrogen; under this concentration, the plant's photorespiration can be neglected, and this serves as the treatment group) and 21% (21% oxygen and 79% nitrogen; this is the atmospheric oxygen concentration, and it serves as the control group). The high-pressure gas cylinders are first connected to a self-made buffer bag. A small amount of water is injected into the buffer bag to simulate the relative humidity in the atmosphere. After passing through the buffer bag, the mixed gas enters the leaf chamber through the intake pipe of the photosynthesis instrument, ensuring the stability of the oxygen concentration and appropriate humidity. Currently, this buffer device has been granted a Chinese national utility model patent (ZL 2015 2 0174847.1). The CO_2 injection system was calibrated using an open gas path with a flow rate of $500 \mu\text{mol}\cdot\text{s}^{-1}$. Before measuring the A_n-C_a and A_n-C_i curves of the two plants, the light response curves of photosynthesis (A_n-I curves, where I is light intensity) for these two plants were measured first. When measuring the A_n-I curve, the CO_2 injection system provided a stable CO_2 concentration. Based on measurements of atmospheric CO_2 concentration, the CO_2 concentration in the instrument chamber was set to $420 \mu\text{mol}\cdot\text{mol}^{-1}$, and the light intensity gradient was set to: 2,000, 1,800, 1,600, 1,400, 1,200, 1,000, 800, 600, 400, 200, 150, 100, 80, 50, $0 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. All measurements used an automatic measurement program, simultaneously recording leaf gas exchange and chlorophyll fluorescence parameters. During automatic measurement, the minimum waiting time for each recording was 2 minutes, and the maximum waiting time was 3 minutes. Before data recording, the instrument automatically performed matching between the reference chamber and the sample chamber. After the data were measured, the "Photosynthesis Model Fitting Software (PMSS)" at

<http://photosynthetic.sinaapp.com/calc.html> (Shenzhen Baoying Technology Computing Co., Ltd., China) was used to fit the A_n-I curves of the two plants in accordance with the photosynthesis light-response model (Ye et al., 2013). The saturated light intensity for both plants was found to be $2,000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

While measuring the A_n-C_a and A_n-C_i curves of the two plants, the light intensity was set to $2,000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and the O_2 concentrations were 2% and 21%, respectively. The CO_2 injection system provided different external CO_2 gradients (C_a): 1,600, 1,400, 1,200, 1,000, 800, 600, 420, 300, 200, 100, 50, and $0 \mu\text{mol}\cdot\text{mol}^{-1}$. All measurements used an automatic measurement program, simultaneously recording leaf gas exchange and chlorophyll fluorescence parameters. During the process of automatic measurement, the waiting time for each recording ranged from a minimum of 2 minutes to a maximum of 3 minutes. Before data recording, the instrument automatically performed matching between the reference chamber and the sample chamber. For the FvCB model, the A_n-C_i curves of the two plant species were meticulously fitted using a method developed by Sharkey et al. (2007) to estimate parameters such as V_{cmax} , $J_{\text{A-max}}$, V_{TPU} , Γ , and R_{day} . In particular, the R_{day} value is derived under the assumption that it equals $0.015 V_{\text{cmax}}$. This method developed by Sharkey et al. is integrated into the Photosynthesis Model Simulation Software (PMSS) platform, which can be accessed at <http://photosynthetic.sinaapp.com/calc.html> (Shenzhen Baoying Technology Computing Co., Ltd., China). Moreover, to determine the $J_{\text{f-max}}$ which represents the maximum electron transport rate associated with Photosystem II (PSII), a comprehensive analysis of the $J-C_i$ curves obtained through measurements is necessary. The $J_{\text{f-max}}$ identified as the uppermost threshold of electron transport rate within these curves, is a crucial parameter for assessing the photosynthetic potential of plants. Its significance becomes particularly pronounced after the $J-C_i$ curves have been meticulously quantified and analyzed.

Additionally, the parameters A_{max} , C_{TPU} , Γ , R_{pa0} , R_{p0} , Γ^* , and R_p can be determined using Model I and Model II with the assistance of the PMSS platform. The PMSS platform offers a user-friendly interface for conducting simulations and extracting key parameters that are essential for analyzing the photosynthetic performance of plants. It is a valuable tool for researchers and students alike. Please be aware that there might be temporary network issues that could prevent the webpage from loading.

2.6 Statistical analysis

All variables were presented as mean values and standard error (mean $\pm$ standard error, $n = 3$) with three replicates. Data were analyzed with one-way analysis of variance (ANOVA). A paired-sample t -test was used to compare whether there was a significant difference between the fitting results and the corresponding observed values at the 5% significance level ($p < 0.05$). The data analysis was performed using the SPSS 18.5 statistical software package (SPSS, Chicago, Illinois, USA). One paired-sample t test

was employed to compare whether there were significant differences between the fitting results and corresponding observed values at the 5% level of significance ($p < 0.05$) using the statistical package of SPSS 18.5 (SPSS, Chicago, IL, United States). Graphs were created using Origin 2021, and final graphic processing was performed with Adobe Illustrator CS5. The determination coefficient (R^2) was used to indicate the degree of fit between the model and observed points, which was calculated as $R^2 = 1 - \text{SSE}/\text{SST}$, where SST is the total sum of squares and SSE is the sum of squared errors.

3 Results

3.1 A_n – C_a and A_n – C_i curves and their fitting with the FvCB model at 21% O_2 concentration

Figure 1 shows the A_n – C_a curves and A_n – C_i curves of *L. perenne* and *T. aestivum* under the conditions of 21% O_2 concentration. As

analyzed in Figure 1, the FvCB model shows significant differences when fitting the A_n – C_a curves and A_n – C_i curves of these two plants.

Specifically, (1) after fitting the A_n – C_a curve of *T. aestivum* with Equations 1, 2, the obtained curve has a large deviation from the actual observed data (Figure 1B); (2) as showed in Figure 1A, the transition point from Rubisco limitation to RuBP limitation ($C_{i, \text{tr}}$) is approximately $1,200 \mu\text{mol} \cdot \text{mol}^{-1}$, which is significantly higher than the currently accepted empirical range of 300 – $600 \mu\text{mol} \cdot \text{mol}^{-1}$, while the curve of *T. aestivum* does not show $C_{i, \text{tr}}$ (Figure 1B), indicating a significant discrepancy from the theory that $C_{i, \text{tr}}$ must exist. Furthermore, when the FvCB model fits the A_n – C_i curves of the two plants, it reveals three key processes affecting C_3 plant carbon assimilation: Rubisco limitation, RuBP regeneration limitation, and TPU limitation (Figures 1C, D).

Table 1 shows the key parameters obtained by fitting the A_n – C_a curves and A_n – C_i curves of *L. perenne* and *T. aestivum* with the FvCB model, including $J_{A-\text{max}}$, V_{cmax} , V_{TPU} , Γ^* , and R_{day} . According to the results in Table 1, the V_{cmax} and V_{TPU} predicted by the FvCB model are two indirect parameters that currently cannot be directly

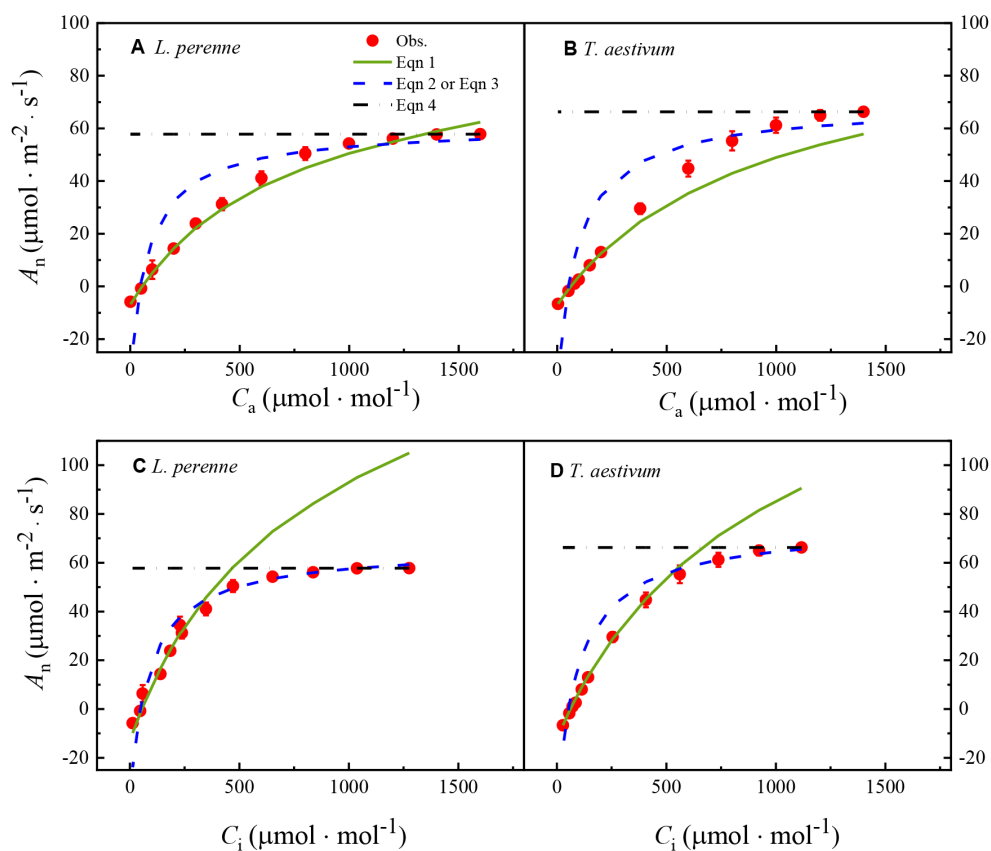


FIGURE 1

The A_n – C_a and A_n – C_i curves for *L. perenne* and *T. aestivum* under atmospheric conditions with an oxygen concentration of 21%. The curves have been fitted with the FvCB model, a comprehensive model that describes the photosynthetic process in C_3 plants, taking into account the carboxylation efficiency of Rubisco, the rate of electron transport, and the triose phosphate utilization. The solid red dots on the curves represent the observed experimental data, which are the actual measurements obtained from the plants under controlled conditions. Each data point is expressed as the mean $\pm$ standard error (SE), and the experiments were conducted with three replicates ($n = 3$) to ensure the robustness and reproducibility of the results.

measured by experiments; Γ^* and R_{day} are two parameters that are difficult to accurately measure in experiments, so there are no corresponding observed values in this study. Among these parameters, only the model predicted value of J_{max} can be directly compared with the actual observed value.

However, the data presented in Table 1 indicate that the $J_{A-\text{max}}$ values derived from fitting the A_n-C_a curve of *L. perenne* using the FvCB model is considerably lower than the observed $J_{f-\text{max}}$ value. In contrast, the $J_{A-\text{max}}$ value obtained by fitting the A_n-C_i curve of *L. perenne* aligns closely with the observed $J_{f-\text{max}}$ value, with no significant discrepancy between the estimated and observed data. Conversely, for *T. aestivum*, the $J_{A-\text{max}}$ value obtained from fitting the A_n-C_a curve is close to the observed $J_{f-\text{max}}$ value.

In addition, the results in Table 1 show that the V_{cmax} values obtained by fitting the A_n-C_a curves with the FvCB model are smaller than the values obtained by fitting the A_n-C_i curves with the model by 46.98% and 44.37% for *L. perenne* and *T. aestivum*, respectively. A similar pattern is observed for the estimation of R_{day} . That is, the R_{day} values obtained by fitting the A_n-C_a curves with the FvCB model are significantly smaller than those obtained by fitting the A_n-C_i curves with the same model, with reductions of 47.10% and 44.30% for *L. perenne* and *T. aestivum*, respectively.

3.2 A_n-C_a and A_n-C_i curves and their fitting with the FvCB model at 2% O_2 concentration

Figure 2 shows the A_n-C_a curves and A_n-C_i curves of *L. perenne* and *T. aestivum* under the conditions of 2% O_2 concentration. As the data presented in Figure 2, it can be observed that the fitted curves using the FvCB model show significant differences in the values of $C_{i,\text{tr}}$. Specifically, the FvCB model predicts significantly higher $C_{i,\text{tr}}$ values when fitting the A_n-C_a curves of *L. perenne* and *T. aestivum* compared to when fitting their A_n-C_i curves (Figure 2). Furthermore, the fitting results of the FvCB model reveal three key biochemical processes affecting C_3 plant carbon assimilation: Rubisco enzyme limitation process, RuBP regeneration limitation, and TPU limitation. These processes play a crucial role in plant photosynthesis under varying oxygen concentration conditions (Figures 2C, D). At 2% O_2 concentration, these limitation processes may differ from those at 21% O_2 concentration, possibly due to plant's adaptive regulation in low-oxygen environments. For example, Rubisco enzyme limitation may be more prominent as oxygen competes with CO_2 for Rubisco's active site, and this competition may be intensified in low-oxygen conditions. At the same time, the regeneration of RuBP and TPU limitation may also be affected by the reduction of oxygen concentration, thereby affecting the overall efficiency of photosynthesis.

Table 2 shows the key parameters obtained by fitting the A_n-C_a and A_n-C_i curves of *L. perenne* and *T. aestivum* with the FvCB model, including $J_{A-\text{max}}$, V_{cmax} , V_{TPU} , Γ^* , and R_{day} . Similar to the results in Table 1, the four parameters V_{cmax} , V_{TPU} , Γ^* , and R_{day} have no corresponding observed values. Only the model-predicted value of $J_{A-\text{max}}$ can be directly compared with the observed $J_{f-\text{max}}$ value. Furthermore, as showed in Table 2, regardless of whether it is the A_n-C_a curves or the A_n-C_i curves of *L. perenne* and *T. aestivum*

TABLE 1 Observed data and results estimated by FvCB model for two C_3 species at 21% O_2 concentration (mean $\pm$ SE, $n = 3$).

	<i>L. perenne</i>				<i>T. aestivum</i>				PD (%)	
	<i>A_n</i> - <i>C_a</i>		<i>A_n</i> - <i>C_i</i>		PD (%)	<i>A_n</i> - <i>C_a</i>		<i>A_n</i> - <i>C_i</i>		
		FvCB model	Obs.	FvCB model	Obs.		FvCB model	Obs.		FvCB model
<i>V_{cmax}</i>	91.52 ± 6.89 ^b	–	172.63 ± 10.57 ^a	–	46.98	110.63 ± 4.53 ^b	–	198.85 ± 3.23 ^a	–	44.37
<i>J_{A,max}</i>	246.39 ± 3.55 ^b	283.85 ± 3.36 ^a	276.18 ± 7.20 ^a	283.85 ± 3.36 ^a	10.79	283.86 ± 6.66 ^a	293.78 ± 3.13 ^a	316.53 ± 5.42 ^a	293.78 ± 3.13 ^b	10.32
<i>V_{TPU}</i>	19.72 ± 0.32 ^a	–	20.13 ± 0.34 ^a	–	2.04	22.65 ± 0.33 ^a	–	23.78 ± 0.35 ^a	–	4.75
<i>Γ</i> *	41.77 ± 2.69 ^a	–	41.77 ± 2.69 ^a	–	0	22.39 ± 0.98 ^a	–	20.54 ± 0.81 ^a	–	9.01
<i>R_{day}</i>	1.37 ± 0.10 ^b	–	2.59 ± 0.16 ^a	–	47.10	1.66 ± 0.07 ^b	–	2.98 ± 0.05 ^a	–	44.30
<i>R</i> ²	0.991	–	0.986	–	0.51	0.987	–	0.999	–	1.20

Estimated and observed parameter values within one plant which are statistically significant different ($p < 0.05$) are annotated with different superscript letter (e.g. 283.85 $\pm$ 3.36^a and 246.34 $\pm$ 5.48^b indicates a significant difference), whereas those which are not statistically significant different ($p > 0.05$) are annotated with the same superscript letter (e.g. 276.18 $\pm$ 7.20^a and 283.85 $\pm$ 3.36^a indicates no significant difference). Percentage differences (PD, %) = (Value derived from A_n-C_i curves - Value derived from A_n-C_a curves) / Value derived from A_n-C_i curves $\times$ 100%. The PD value is represented by its absolute value. The unit of V_{cmax} , V_{TPU} , and R_{day} is $\mu\text{mol m}^{-2} \text{s}^{-1}$; the unit of Γ^* is $\mu\text{mol mol}^{-1}$.

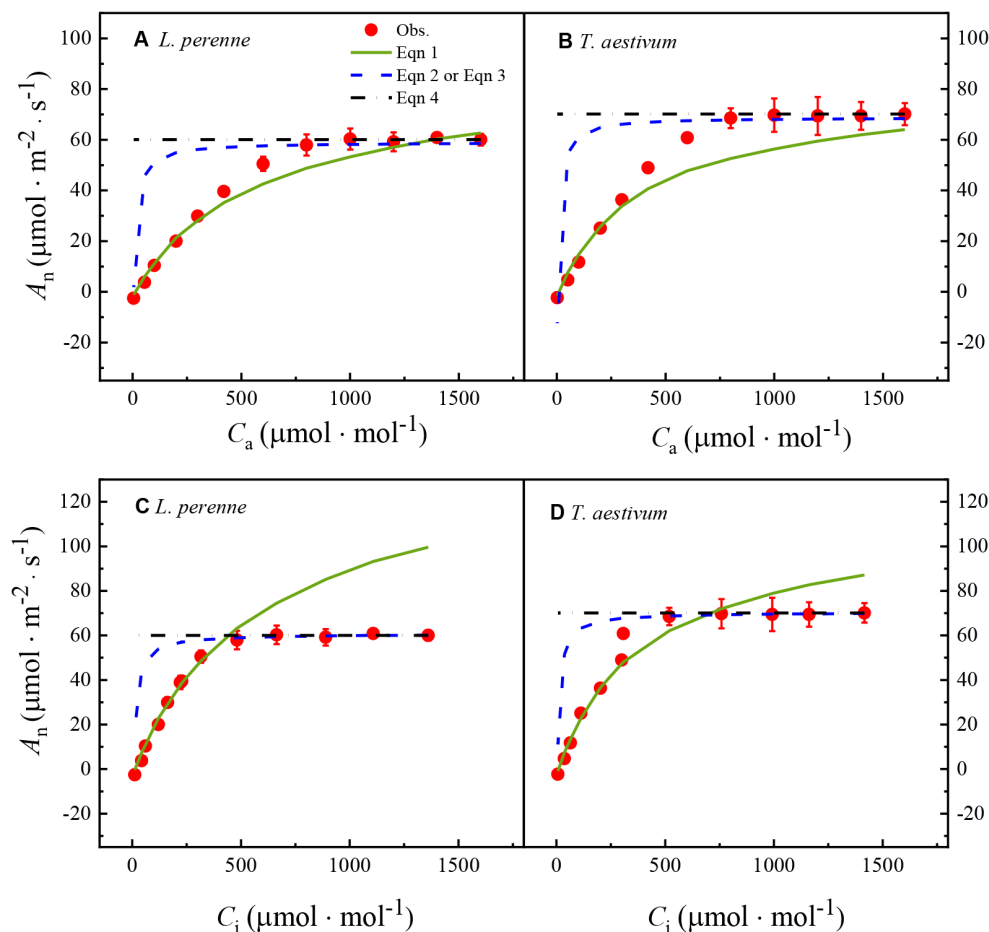


FIGURE 2

The A_n – C_a and A_n – C_i curves for *L. perenne* and *T. aestivum* under a reduced oxygen concentration of 2%. The curves have been fitted with the FvCB model, a comprehensive model that describes the photosynthetic process in C_3 plants, taking into account the carboxylation efficiency of Rubisco, the rate of electron transport, and the triose phosphate utilization. The solid red dots on the curves represent the observed experimental data, which are the actual measurements obtained from the plants under controlled conditions. Each data point is expressed as the mean $\pm$ standard error (SE), and the experiments were conducted with three replicates ($n = 3$) to ensure the robustness and reproducibility of the results.

fitted with the FvCB model, the derived $J_{A-\max}$ values closely approximate the observed $J_{f-\max}$ value, and there is no significant difference ($p > 0.05$) between the estimated values and the observed data (Table 2).

In addition, the results in Table 2 also show that the $V_{\max}$ values obtained by fitting the A_n – C_a curves with the FvCB model are significantly smaller ($p < 0.05$) than the values obtained by fitting the A_n – C_i curves with the model by 46.63% and 37.66% for *L. perenne* and *T. aestivum*, respectively. A similar situation applies to the estimation of R_{day} . That is, the R_{day} values derived by fitting the A_n – C_a curves with the FvCB model are significantly lower ($p < 0.05$) than those obtained by fitting the A_n – C_i curves with the same model, with reductions of 47.03% and 37.36% for *L. perenne* and *T. aestivum*, respectively. This discrepancy underscores the importance of the curve types in fitting, as it can influence estimated parameters and, in turn, our understanding of the photosynthetic responses in different plant species. Furthermore,

the Γ_* values estimated by the FvCB model for both plant species appear to be underestimated (Table 2).

3.3 A_n – C_a and A_n – C_i curves and their fitting using the Model I or Model II at 21% O_2 concentration

Figure 3 shows the A_n – C_a and A_n – C_i curves of *L. perenne* and *T. aestivum*, along with the fitting curves generated by Model I or Model II. As shown in Figure 3, both models can accurately reproduce these plant's A_n – C_a and A_n – C_i curves at 21% O_2 concentration, with the determination coefficient (R^2) at least 0.995.

Table 3 presents several parameters derived from fitting the A_n – C_a and A_n – C_i curves of *L. perenne* and *T. aestivum* using Model I or Model II, including α_0 , $A_{\max}$, $C_{i,TPU}$, Γ_* , Γ , R_{p0} , and R_{pao} . The data in Table 3 indicate that the $A_{\max}$ and Γ values obtained by fitting the

TABLE 2 Observed data and results estimated by FvCB model for two C₃ species at 2% O₂ concentration (mean ± SE, n = 3).

	<i>L. perenne</i>					<i>T. aestivum</i>				PD (%)
	<i>A_n-C_a</i>		<i>A_n-C_i</i>		PD (%)	<i>A_n-C_a</i>		<i>A_n-C_i</i>		
	FvCB model	Obs.	FvCB model	Obs.		FvCB model	Obs.	FvCB model	Obs.	
<i>V_{cmax}</i>	71.67 ± 5.56 ^b	–	134.29 ± 11.22 ^a	–	46.63	72.48 ± 7.14 ^b	–	116.27 ± 8.52 ^a	–	37.66
<i>J_{A-max}</i>	240.08 ± 6.55 ^a	246.34 ± 5.48 ^a	251.78 ± 6.71 ^a	246.34 ± 5.48 ^a	4.65	279.63 ± 11.75 ^a	252.46 ± 6.90 ^a	289.66 ± 14.38 ^a	252.46 ± 6.90 ^a	3.46
<i>V_{TPU}</i>	20.37 ± 0.42 ^a	–	20.68 ± 0.40 ^a	–	1.50	23.74 ± 0.87 ^a	–	23.96 ± 0.87 ^a	–	0.92
<i>Γ*</i>	4.42 ± 0.31 ^a	–	4.42 ± 0.31 ^a	–	0	3.84 ± 0.21 ^a	–	3.84 ± 0.21 ^a	–	0
<i>R_{day}</i>	1.07 ± 0.08 ^b	–	2.02 ± 0.17 ^a	–	47.03	1.09 ± 0.11 ^b	–	1.74 ± 0.13 ^a	–	37.36

Estimated and observed parameter values within one plant which are statistically significant different ($p < 0.05$) are annotated with different superscript letter (e.g. 272.39 ± 4.89^a and 246.34 ± 5.48^b indicates a significant difference), whereas those which are not statistically significant different ($p > 0.05$) are annotated with the same superscript letter (e.g. 20.70 ± 0.41^a and 20.70 ± 0.41^a indicates no significant difference). Percentage differences (PD, %) = (Value derived from *A_n-C_i* curves – Value derived from *A_n-C_a* curves) / Value derived from *A_n-C_i* curves × 100%. The PD value is represented by its absolute value. The unit of *V_{cmax}*, *J_{A-max}*, *V_{TPU}*, and *R_{day}* is μmol m⁻² s⁻¹; the unit of *Γ** is μmol mol⁻¹.

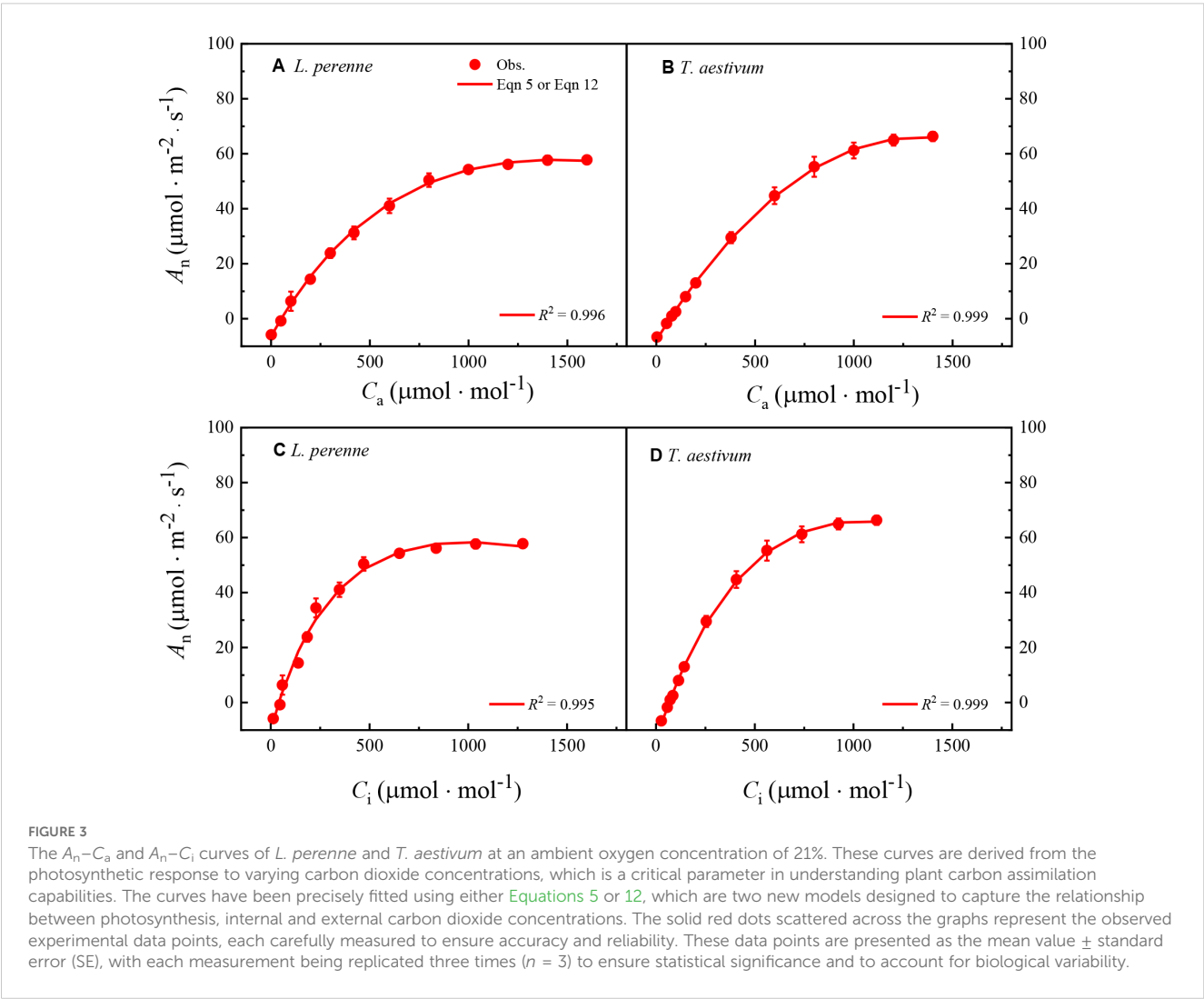


FIGURE 3 The A_n-C_a and A_n-C_i curves of *L. perenne* and *T. aestivum* at an ambient oxygen concentration of 21%. These curves are derived from the photosynthetic response to varying carbon dioxide concentrations, which is a critical parameter in understanding plant carbon assimilation capabilities. The curves have been precisely fitted using either Equations 5 or 12, which are two new models designed to capture the relationship between photosynthesis, internal and external carbon dioxide concentrations. The solid red dots scattered across the graphs represent the observed experimental data points, each carefully measured to ensure accuracy and reliability. These data points are presented as the mean value ± standard error (SE), with each measurement being replicated three times ($n = 3$) to ensure statistical significance and to account for biological variability.

A_n - C_a curves of *L. perenne* and *T. aestivum* with both models do not significantly differ ($p > 0.05$) from those obtained from the A_n - C_i curves. The R_{pa0} values derived from fitting the A_n - C_a curves of *L. perenne* and *T. aestivum* with Model I or Model II are close to their respective observed values, with no significant difference ($p > 0.05$) between them (Table 3). However, the R_{pa0} values obtained by fitting the A_n - C_i curves of *L. perenne* and *T. aestivum* with either Model I or Model II are considerably higher than the corresponding observed values, exhibiting a significant discrepancy ($p < 0.05$, Table 3). Furthermore, for *L. perenne* and *T. aestivum* at 21% O_2 conditions, the values of α_0 derived from A_n - C_a curves estimated by Model I were 57.09% and 52.26% lower than those obtained by fitting their A_n - C_i curves (Table 3).

3.4 A_n - C_a and A_n - C_i curves and their fitting using the Model I or Model II at 2% O_2 concentration

Figure 4 shows the A_n - C_a and A_n - C_i curves of *L. perenne* and *T. aestivum* at 2% O_2 concentration, along with the fitting curves generated by Model I or Model II, with the R^2 at least 0.989. As showed in Figure 4, both models can accurately reproduce the A_n - C_a and A_n - C_i curves of these two plants at 2% O_2 concentration.

Table 4 shows the parameters obtained by fitting the A_n - C_a and A_n - C_i curves of *L. perenne* and *T. aestivum* with Model I and Model II at 2% O_2 concentration, including α_0 , A_{max} , $C_{i,TPU}$, Γ , Γ^* , R_{p0} , and R_{pa0} . The data in Table 4 indicate that, similar to the results in Table 3, for

TABLE 3 The observed data and the outcomes generated by Model I and Model II for the two C_3 species at a 21% O_2 concentration for A_n - C_a and A_n - C_i curves are presented (mean $\pm$ SE, $n = 3$).

	<i>L. perenne</i>					<i>T. aestivum</i>				PD (%)	
	<i>A_n</i> - <i>C_a</i>		<i>A_n</i> - <i>C_i</i>			PD (%)	<i>A_n</i> - <i>C_a</i>		<i>A_n</i> - <i>C_i</i>		
	Model I	Obs.	Model I	Obs.			Model I	Obs.	Model I		Obs.
<i>α</i> ₀	0.127 ± 0.007 ^b	-	0.296 ± 0.018 ^a	-	57.09	0.116 ± 0.004 ^b	-	0.243 ± 0.007 ^a	-	52.26	
<i>A</i> _{max}	58.12 ± 0.73 ^a	57.80 ± 0.88 ^a	58.66 ± 0.61 ^a	57.80 ± 0.88 ^a	0.92	66.44 ± 1.15 ^a	66.28 ± 0.98 ^a	66.29 ± 1.14 ^a	66.28 ± 0.98 ^a	0.23	
<i>C_i</i> _{TPU}	1441.12 ± 87.03 ^a	1533.26 ± 66.72 ^a	908.91 ± 36.60 ^a	1075.66 ± 120.47 ^a	58.55	1357.05 ± 63.31 ^a	1399.91 ± 0.16 ^a	1006.74 ± 29.46 ^a	1072.49 ± 32.85 ^a	34.80	
<i>Γ</i>	49.91 ± 8.35 ^a	55.93 ± 4.63 ^a	47.85 ± 8.39 ^a	53.75 ± 4.22 ^a	4.31	69.29 ± 1.92 ^a	69.36 ± 1.63 ^a	66.52 ± 0.37 ^a	66.83 ± 0.48 ^a	4.16	
<i>Γ</i> *	38.76 ± 7.39 ^a	–	31.03 ± 5.86 ^a	–	24.91	40.24 ± 2.09 ^b	–	51.57 ± 1.13 ^a	–	21.97	
<i>R</i> _{p0}	3.88 ± 0.68 ^b	–	10.79 ± 2.20 ^a	–	64.04	4.50 ± 0.40 ^b	–	11.68 ± 0.07 ^a	–	61.47	
<i>R</i> _{day}	2.59 ± 0.16 ^a	–	2.59 ± 0.16 ^a	-	0	3.09 ± 0.08 ^a	–	3.09 ± 0.08 ^a	–	0	
<i>R</i> ²	0.996	–	0.995	–	0.10	0.999	–	0.999	–	0	
	<i>A_n</i> - <i>C_a</i>		<i>A_n</i> - <i>C_i</i>		PD (%)	<i>A_n</i> - <i>C_a</i>		<i>A_n</i> - <i>C_i</i>		PD (%)	
	Model II	Obs.	Model II	Obs.		Model II	Obs.	Model II	Obs.		
<i>α</i> ₀	0.127 ± 0.007 ^b	-	0.296 ± 0.018 ^a	-	57.09	0.116 ± 0.004 ^b	-	0.243 ± 0.007 ^a	-	52.26	
<i>A</i> _{max}	58.12 ± 0.73 ^a	57.80 ± 0.88 ^a	58.66 ± 0.61 ^a	57.80 ± 0.88 ^a	0.92	66.44 ± 1.15 ^a	66.28 ± 0.98 ^a	66.29 ± 1.14 ^a	66.28 ± 0.98 ^a	0.23	
<i>C_i</i> _{TPU}	1441.12 ± 87.03 ^a	1533.26 ± 66.72 ^a	908.91 ± 36.60 ^a	1075.66 ± 120.47 ^a	58.55	1357.05 ± 63.31 ^a	1399.91 ± 0.16 ^a	1006.74 ± 29.46 ^a	1072.49 ± 32.85 ^a	34.80	
<i>Γ</i>	49.91 ± 8.35 ^a	55.93 ± 4.63 ^a	47.85 ± 8.39 ^a	53.75 ± 4.22 ^a	4.31	69.29 ± 1.92 ^a	69.36 ± 1.63 ^a	66.52 ± 0.37 ^a	66.83 ± 0.48 ^a	4.16	
<i>R</i> _{pa0}	6.47 ± 0.67 ^a	5.82 ± 0.49 ^a	13.38 ± 2.17 ^a	5.82 ± 0.49 ^b	51.64	7.76 ± 0.28 ^a	7.21 ± 0.11 ^a	14.77 ± 0.01 ^a	7.21 ± 0.11 ^b	47.46	
<i>R</i> ²	0.996	–	0.995	–	0.10	0.999	–	0.999	–	0	

For a single plant, calculated and observed parameter values that exhibit a statistically significant difference ($p < 0.05$) are marked with distinct superscript letters. For instance, 0.127 ± 0.007^b and 0.296 ± 0.018^a signify a significant discrepancy. Conversely, values that do not show a statistically significant difference ($p > 0.05$) are denoted with the same superscript letter, indicating no significant variation. An example of this would be 58.12 ± 0.73^a and 57.80 ± 0.88^a , which suggest no significant difference. Percentage differences (PD, %) = (Value derived from A_n - C_i curves - Value derived from A_n - C_a curves) / Value derived from A_n - C_i curves $\times$ 100%. The PD value is represented by its absolute value. The unit of A_{max} , R_{p0} , R_{pa0} and R_{day} is $\mu\text{mol m}^{-2} \text{s}^{-1}$; the unit of Γ , Γ^* and $C_{i,TPU}$ is $\mu\text{mol mol}^{-1}$. It is important to highlight that a comparative analysis has been conducted in Table 3, examining the parameters Γ , R_{p0} and R_{day} between A_n - C_a and A_n - C_i curves to ascertain whether there are significant differences between these parameters.

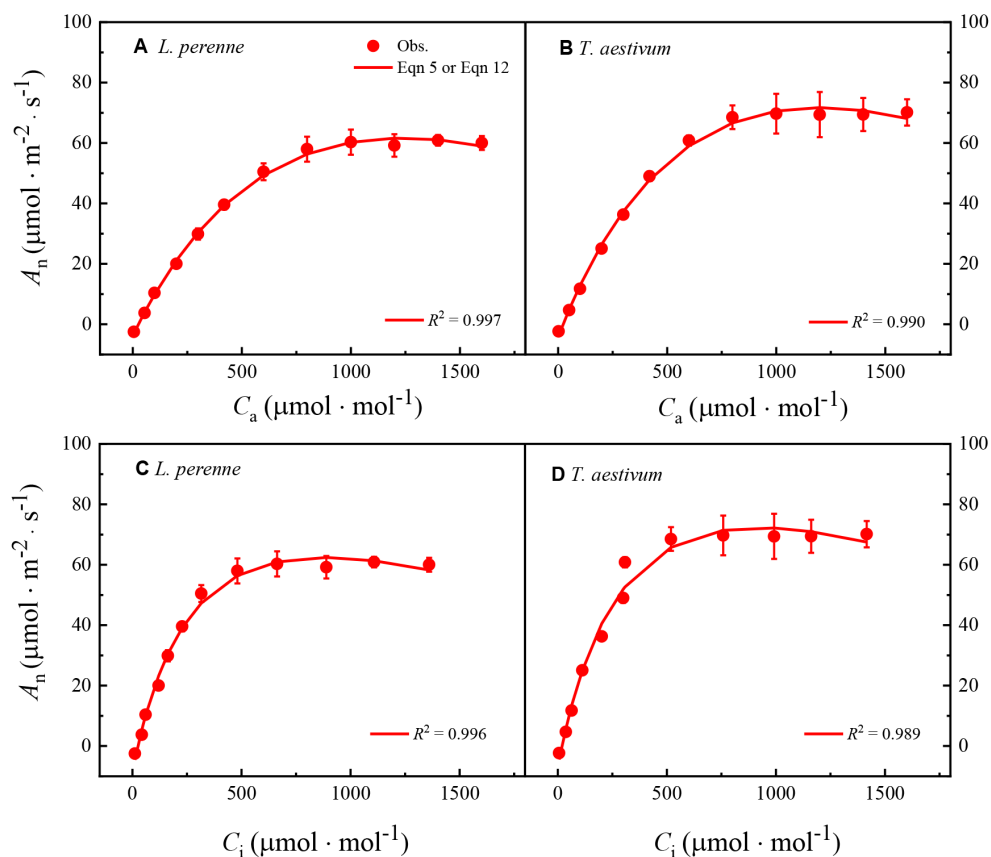


FIGURE 4

The A_n – C_a and A_n – C_i response curves for both *L. perenne* and *T. aestivum* under an oxygen concentration of 2%. These curves have been meticulously fitted using either Equations 5 or 12, which is indicated in the respective graphs. The solid red dots scattered across the curves correspond to the observed experimental data points, providing a visual representation of the actual measurements taken during the study. Each data point is presented as the mean value, with error bars indicating the standard error of the mean (SEM) to convey the variability within the dataset. It is important to note that the data are based on three replicates ($n = 3$), ensuring a robust statistical foundation for the analysis.

both the A_n – C_a and A_n – C_i curves, the A_{max} and Γ values obtained from fitting with the two models do not significantly differ ($p > 0.05$) from the corresponding observed values (except for the Γ values of *T. aestivum* obtained from the A_n – C_a curve fitting with Model II). In addition, for *L. perenne*, the R_{pa0} value obtained by fitting the A_n – C_a curve with Model II is close to the observed value, and there is no significant difference ($p > 0.05$) between the two (Table 4). On the contrary, when Model II is employed to fit the A_n – C_i curves of *L. perenne* and *T. aestivum*, the R_{pa0} value obtained is significantly higher ($p < 0.05$) than the corresponding observed value (Table 4). Moreover, we found that there is a significant difference ($p < 0.05$) between the R_{pa0} value calculated by Model II and the observed data at 2% O_2 concentration for *T. aestivum*. To avoid potential issues with the results, we opted to use Model II for fitting the A_n – C_a curve within the range of 0 to 300 $\mu\text{mol}\cdot\text{mol}^{-1}$. By doing this, the R_{pa0} value calculated with Model II is $(2.45 \pm 0.15) \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, which shows no significant difference when compared to the observed value of $(2.31 \pm 0.17) \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. In this instance, the coefficient of determination (R^2) exceeds 0.9995, signifying an exceptionally high level of agreement between the fitted and observed data points. For *L.*

perenne, following the same procedures, we determined the R_{pa0} value using Model II to be $(3.09 \pm 0.38) \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. This value is in close proximity to the observed value, which is $(3.03 \pm 0.23) \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, with no significant divergence between them. When compared with the initial value of $(3.68 \pm 0.41) \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ presented in Table 4, the calculated value of $(3.09 \pm 0.38) \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ is more closely aligned with the observed values. Here as well, with an R^2 value surpassing 0.9977, we observe a remarkable alignment between the model's predictions and the empirical data, reinforcing the model's efficacy and reliability.

It is noteworthy that there is a significant difference ($p < 0.05$) in the α_0 values obtained by fitting the A_n – C_a and A_n – C_i curves of *L. perenne* and *T. aestivum* with the Model I. The values of α_0 derived from A_n – C_a curves estimated by Model I were 63.41% and 51.79% lower than those obtained by fitting their A_n – C_i curves for *L. perenne* and *T. aestivum* at 2% O_2 conditions. This finding may indicate that the model is sensitive to different CO_2 response curves. Furthermore, when comparing Tables 2, 4, it can be observed that the Γ^* values derived from Model I are higher than those estimated by the FvCB model for both plant species.

TABLE 4 The observed data and the outcomes generated by Model I and Model II for the two C₃ species at a 2% O₂ concentration for A_n-C_a and A_n-C_i curves are presented (mean ± SE, n = 3).

	<i>L. perenne</i>					<i>T. aestivum</i>				PD (%)	
	<i>A_n-C_a</i>		<i>A_n-C_i</i>			PD (%)	<i>A_n-C_a</i>		<i>A_n-C_i</i>		
	Model I	Obs.	Model I	Obs.			Model I	Obs.	Model I		Obs.
α_0	0.150 ± 0.001 ^b	-	0.410 ± 0.035 ^a	-	63.41	0.189 ± 0.006 ^b	-	0.392 ± 0.012 ^a	-	51.79	
$A_{\max}$	61.72 ± 1.92 ^a	63.10 ± 2.07 ^a	61.63 ± 1.75 ^a	63.10 ± 2.07 ^a	0.15	71.83 ± 3.43 ^a	71.18 ± 3.44 ^a	72.61 ± 3.72 ^a	71.18 ± 3.44 ^a	1.07	
$C_{i,TPU}$	1245.12 ± 28.85 ^a	1266.55 ± 133.42 ^a	821.27 ± 44.99 ^a	895.56 ± 162.09 ^a	51.61	1197.18 ± 6.35 ^a	1400.58 ± 115.97 ^a	846.74 ± 36.82 ^a	1077.12 ± 190.73 ^a	41.39	
Γ	25.22 ± 3.02 ^a	24.74 ± 3.53 ^a	24.94 ± 2.61 ^a	23.79 ± 3.65 ^a	1.12	22.39 ± 0.98 ^a	17.92 ± 1.11 ^b	20.54 ± 0.81 ^a	17.36 ± 1.17 ^a	9.01	
Γ^*	17.70 ± 2.36 ^a	-	21.84 ± 2.47 ^a	-	18.96	16.35 ± 1.66 ^a	-	15.61 ± 1.17 ^a	-	4.74	
R_{p0}	2.60 ± 0.32 ^b	-	7.45 ± 1.40 ^a	-	65.10	3.05 ± 0.38 ^b	-	5.86 ± 0.55 ^a	-	47.95	
R_{day}	1.07 ± 0.08 ^b	-	2.02 ± 0.17 ^a	-	47.03	1.09 ± 0.11 ^b	-	1.74 ± 0.13 ^a	-	37.36	
R^2	0.997	-	0.990	-	0.71	0.996	-	0.989	-	0.71	
	<i>A_n-C_a</i>		<i>A_n-C_i</i>		PD (%)	<i>A_n-C_a</i>		<i>A_n-C_i</i>		PD (%)	
	Model II	Obs.	Model II	Obs.		Model II	Obs.	Model II	Obs.		
α_0	0.150 ± 0.001 ^b	-	0.410 ± 0.035 ^a	-	63.41	0.189 ± 0.006 ^b	-	0.392 ± 0.012 ^a	-	51.79	
$A_{\max}$	61.72 ± 1.92 ^a	63.10 ± 2.07 ^a	61.63 ± 1.75 ^a	63.10 ± 2.07 ^a	0.15	71.83 ± 3.43 ^a	71.18 ± 3.44 ^a	72.61 ± 3.72 ^a	71.18 ± 3.44 ^a	1.07	
$C_{i,TPU}$	1245.12 ± 28.85 ^a	1266.55 ± 133.42 ^a	821.27 ± 44.99 ^a	895.56 ± 162.09 ^a	51.61	1197.18 ± 6.35 ^a	1400.58 ± 115.97 ^a	846.74 ± 36.82 ^a	1077.12 ± 190.73 ^a	41.39	
Γ	25.22 ± 3.02 ^a	24.74 ± 3.53 ^a	24.94 ± 2.61 ^a	23.79 ± 3.65 ^a	1.12	22.39 ± 0.98 ^a	17.92 ± 1.11 ^b	20.54 ± 0.81 ^a	17.36 ± 1.17 ^a	9.01	
R_{pa0}	3.68 ± 0.41 ^a	3.03 ± 0.23 ^a	9.47 ± 1.56 ^a	3.03 ± 0.23 ^b	61.14	3.81 ± 0.33 ^a	2.31 ± 0.17 ^b	7.61 ± 0.44 ^a	2.31 ± 0.17 ^b	49.93	
R^2	0.997	-	0.990	-	0.71	0.996	-	0.989	-	0.71	

For a single plant, calculated and observed parameter values that exhibit a statistically significant difference ($p < 0.05$) are marked with distinct superscript letters. For instance, 0.150 ± 0.001^b and 0.410 ± 0.035^a signify a significant discrepancy. Conversely, values that do not show a statistically significant difference ($p > 0.05$) are denoted with the same superscript letter, indicating no significant variation. An example of this would be 61.72 ± 1.92^a and 63.10 ± 2.07^a , which suggest no significant difference. Percentage differences (PD, %) = (Value derived from A_n-C_i curves - Value derived from A_n-C_a curves) / Value derived from A_n-C_i curves × 100%. The PD value is represented by its absolute value. The unit of A_{max}, R_{p0}, R_{pa0} and R_{day} is μmol m⁻² s⁻¹; the unit of Γ, Γ* and C_{i,TPU} is μmol mol⁻¹. It is important to highlight that a comparative analysis has been conducted in Table 4, examining the parameters Γ, R_{p0} and R_{day} between A_n-C_a and A_n-C_i curves to ascertain whether there are significant differences between these parameters.

4 Discussions

4.1 The FvCB model in fitting CO₂-response curve of photosynthesis and estimating photosynthetic parameters

In this study, the application of the FvCB model and Model I highlighted the importance of model selection in simulating the process of photosynthesis in C₃ plant species. The differences in the performance of the two models across different oxygen concentrations provided valuable information about the applicability and parameter sensitivity of these models.

The FvCB model is a widely used photosynthesis model in plant physiological research, based on the photosynthetic biochemical mechanism proposed by Farquhar et al. (1980). This model predicts the photosynthetic rate of plants under different environmental conditions by simulating key biochemical processes in photosynthesis, such as the carboxylation reaction of Rubisco, the regeneration of RuBP, and photorespiration. The FvCB model is particularly suitable for analyzing the photosynthetic performance of C₃ plant species under changing environmental conditions, such as different CO₂ concentrations, temperatures, and light intensities (von Caemmerer and Farquhar, 1981; Walker et al., 2017; Miyazawa et al., 2020; Xiao et al., 2021; Yin et al., 2021). In

practical applications, the FvCB model has been used to assess the potential impact of climate change on crop yields (Long and Bernacchi, 2003; Fan et al., 2011) and to study plant adaptability to environmental changes (Morfopoulos et al., 2014; Han et al., 2020). For example, by simulating photosynthesis under different CO₂ concentrations, researchers can predict the future impact of increasing atmospheric CO₂ concentrations on plant growth and ecosystem carbon cycling (Ainsworth and Long, 2005, 2021). In addition, the FvCB model has also been used to optimize agricultural management practices, such as irrigation and fertilization, to improve the light energy utilization efficiency and yield of crops (Zhu et al., 2010; De Kauwe et al., 2016; Vijayakumar et al., 2024).

Although the FvCB model holds significant value in plant physiological ecology research, its accuracy and applicability are still constrained by the model parameterization method and changes in environmental conditions. For example, in this study, fitting the A_n-C_a curves or A_n-C_i curves of plants with the model produced five important photosynthetic parameters: J_{A-max} , V_{cmax} , V_{TPU} , Γ , and R_{day} (Tables 1, 2). However, only the estimated parameter J_{A-max} can be directly compared with the observed J_{f-max} value (Tables 1, 2). At the same time, under normal conditions, the distribution of the electron flow from Photosystem II clearly indicates that the J_{A-max} allocated for carbon assimilation is significantly lower than the J_{f-max} . In the FvCB model, this parameter is not obtained by fitting the electron transport rate to the CO₂ response ($J-C_i$) data, but is indirectly estimated by fitting the A_n-C_a curve or A_n-C_i curve. This may lead to the model overestimating or underestimating the J_{A-max} in plants. In this study, it was found that the J_{A-max} value obtained by fitting the A_n-C_a curve of *L. perenne* with the FvCB model was significantly lower ($p < 0.05$) than the observed value at 21% O₂ concentration (Table 1), whereas the J_{A-max} value obtained by fitting the A_n-C_i curve of *L. perenne* was close to the observed value (Table 1). In contrast, the J_{A-max} values obtained by fitting the A_n-C_a and A_n-C_i curves of *T. aestivum* with the FvCB model showed the opposite trend (Table 1). However, the J_{A-max} value derived from fitting the A_n-C_i curve of *T. aestivum* is markedly higher than the observed J_{f-max} value, indicating a significant divergence between the estimated value and the empirical data (Table 1). Given the starkly contrasting outcomes for J_{A-max} when fitting the A_n-C_a and A_n-C_i curves with the FvCB model, it remains uncertain which response curve is more justified. This phenomenon may be related to the inaccurate simulation of the carboxylation reaction of the key enzyme Rubisco in photosynthesis by the model. Under the condition of 21% O₂ concentration, the oxygenation of Rubisco may be overestimated, resulting in the model's prediction of J_{A-max} being either too high or too low.

On the other hand, as explained by von Caemmerer (2000) and Long and Bernacchi (2003), under 21% O₂ conditions, J_f supports not only J_A but also J_O , J_{Nit} , and J_{MAP} . This relationship can be expressed as $J_f = J_A + J_O + J_{Nit} + J_{MAP}$. Consequently, J_{A-max} must be less than J_{f-max} . Under 2% O₂ conditions, J_O can be neglected. In this case, the relationship can be expressed as $J_f = J_A + J_{Nit} + J_{MAP}$, and J_{A-max} must still be less than J_{f-max} . Based on this criterion, we observed that under

21% O₂ concentration the J_{A-max} estimated by the FvCB model when fitting the A_n-C_i curve of *T. aestivum* exceeds the J_{f-max} (Table 1). Taking into account that J_{Nit} and J_{MAP} are non-zero, we further found that the J_{A-max} estimated by the FvCB model when fitting both the A_n-C_a and A_n-C_i curves of *L. perenne* and *T. aestivum* also surpasses the J_{f-max} under 2% O₂ concentration (Table 2).

Furthermore, the findings in Table 1 reveal a pattern in the V_{cmax} values derived from the FvCB model when it is applied to the A_n-C_a and A_n-C_i curves of *L. perenne* and *T. aestivum*. Specifically, the V_{cmax} values obtained from fitting the A_n-C_a curves are consistently lower than those derived from fitting the A_n-C_i curves for both plant species. A similar trend is observed for the estimation of R_{day} ; that is, the R_{day} values derived from the A_n-C_a curve fits are also consistently lower than those from the A_n-C_i curve fits using the FvCB model for *L. perenne* and *T. aestivum*. Despite these observations, it remains challenging to discern which set of values—those from the A_n-C_a or A_n-C_i curve fits—provides a more accurate representation of the true V_{cmax} and R_{day} values for these two plant species. This uncertainty highlights the complexity of accurately modeling photosynthetic parameters. Thus, there is a need for further investigation to refine our understanding of how different models perform across various plant species and under different physiological conditions.

It is noteworthy that the choice between A_n-C_a and A_n-C_i curve fitting may be influenced by factors such as the atmospheric CO₂ concentration, the specific photosynthetic pathway of the plant, and the presence of other environmental stressors. Therefore, a comprehensive analysis considering these factors and more experimental data may be necessary to determine the most appropriate model for accurately predicting V_{cmax} and R_{day} values. This could involve comparing the FvCB model's predictive power with other models, examining its sensitivity to initial conditions, and assessing its robustness under varying environments.

At 2% oxygen concentration, the FvCB model's fitting results differ significantly from those at the typical atmospheric concentration of 21% O₂ (Figures 1, 2; Tables 1, 2). This discrepancy may indicate the plant's adaptive responses in its photosynthetic machinery under hypoxic conditions. Notably, the model's estimations of J_{A-max} for both *L. perenne* and *T. aestivum* closely approximate the actual observations, regardless of whether the A_n-C_a curve or the A_n-C_i curve is fitted (Table 2). It is important to highlight that the J_{A-max} value predicted by the FvCB model exceeds the observed J_{f-max} value for *T. aestivum* when either the A_n-C_a curve or the A_n-C_i curve is modeled. This outcome is perplexing given the prevailing photosynthetic theory as articulated by von Caemmerer (2000), which posits that J_{A-max} should be less than J_{f-max} . Consequently, the findings are currently challenging to interpret.

Under hypoxic conditions of 2% O₂, the carboxylation efficiency of Rubisco is likely impeded, and photorespiration gains prominence, as indicated by Busch and Sage (2017). The FvCB model reveals that Rubisco limitation, RuBP regeneration limitation, and TPU limitation, which are all pivotal to the photosynthetic process in C₃ plants. Under low O₂ environments, these limitations may become more pronounced and dampen the

overall photosynthetic efficiency, as supported by [Zhu et al. \(2010\)](#). Furthermore, at 21% O₂ (normoxic condition), the FvCB model-calculated V_{cmax} values for the A_n - C_a curve are systematically lower than those for the A_n - C_i curve when analyzing the two plant species ([Table 1](#)). This suggests that photosynthetic parameters are sensitive to oxygen levels, and the FvCB model may require refinements to accurately estimate them under different environmental conditions.

4.2 Model I and Model II in fitting CO₂-response curve of photosynthesis and estimating photosynthetic parameters

In this study, both Model I and Model II exhibited a high R^2 when fitting the A_n - C_a and A_n - C_i curves of *L. perenne* and *T. aestivum*, indicating that the two models have high accuracy in simulating the photosynthesis curves under varying oxygen concentrations ([Figures 3, 4](#)). This result may be attributed to the advantages of the Model I and Model II in parameterization and model structure, enabling them to better capture the photosynthetic response of plants under different environmental conditions. Moreover, both models effectively capture the A_n - C_a and A_n - C_i curves, demonstrating the reduction in carbon assimilation rates under elevated CO₂ concentrations. They also enable the direct calculation of C_{TPU} ([Tables 3, 4](#)). The results of our study show that the models' estimation of the A_{max} and Γ values for *L. perenne* and *T. aestivum* under the two O₂ concentrations does not show significant differences from the corresponding observed values ([Tables 3, 4](#)). Indeed, a notable discrepancy is observed between the R_{pa0} values derived from fitting the A_n - C_a curves and those derived from fitting the A_n - C_i curves, as shown in [Tables 3, 4](#). Despite this, the scientific community has not yet reached a definitive conclusion on which of these R_{pa0} values—obtained from the A_n - C_a curve or the A_n - C_i curve—more accurately represents the true R_{pa0} value. However, considering the nuances of measurement technology, it is plausible to suggest that the R_{pa0} value obtained from the A_n - C_a curve may be closer to the actual R_{pa0} value. This assumption is based on the belief that the R_{pa0} values, particularly those fitted for *L. perenne* and *T. aestivum* using Model II, reflect the true R_{pa0} values for these plant species. This belief is further supported by the A_n - C_a curve's more direct measurement of CO₂ assimilation and its lesser influenced by internal CO₂ concentration changes compared to the A_n - C_i curve. Therefore, the R_{pa0} derived from the A_n - C_a curve is often considered to be more representative of the plant's true photorespiratory rate at CO₂ concentrations approaching zero.

Furthermore, the rationale behind this preference is rooted in the technology used in plant photosynthesis measurement instruments. These instruments utilize non-diffusive infrared CO₂ analysis technology to measure CO₂ concentrations. This method takes advantage of the significant absorption of CO₂ at a specific wavelength of infrared light, a characteristic not shared by O₂. Consequently, it is advisable to use the A_n - C_a curves derived from

such measurements for quantitative studies of plant R_{pa0} . Given this, when conducting research on R_{pa0} in plants, it is more logical to fit the A_n - C_a curves using Model II rather than the A_n - C_i curves. Model II's curve-fitting approach better matches the capabilities of non-diffusive infrared CO₂ analysis technology, making it a more suitable choice for accurate and reliable R_{pa0} assessments. In this study, another notable finding is that once Model I can determine the value of R_{day} , it can also compute the value of R_{p0} ([Tables 3, 4](#)). However, the precision of determining R_{day} presents a challenge, which in turn affects the accuracy of the derived R_{p0} . Nevertheless, our research suggests that when R_{day} can be accurately ascertained, our methodology offers a viable approach for estimating R_{p0} .

4.3 Comparative analysis of the FvCB model and the new models

The FvCB model and Model I/II represent fundamentally divergent paradigms in photosynthetic modeling. As a biochemical mechanistic framework, the FvCB model simulates enzymatic processes governing carbon assimilation ([Farquhar et al., 1980](#)), whereas Model I/II adopts an empirical approach emphasizing practical parameterization. This dichotomy reflects their core objectives: the FvCB model prioritizes biochemical fidelity, while Model I/II emphasizes operational simplicity and environmental adaptability.

Our comparative analysis demonstrates that the superior predictive performance of Models I/II in estimating R_{pa0} and critical photosynthetic parameters (A_{max} , Γ , and C_{TPU}) across oxygen gradients stems from their distinct structural architectures. The FvCB model's dependence on rigid biochemical constraints—including fixed $R_{\text{day}}/V_{\text{cmax}}$ ratios ([von Caemmerer, 2013](#); [De Kauwe et al., 2016](#)), stoichiometric electron requirements (4 e⁻ per CO₂; [Long and Bernacchi, 2003](#)), and invariant photorespiratory CO₂ release (0.5 mol/RuBP oxygenation; [Farquhar et al., 1980](#))—introduces systematic biases. In contrast, Model I dynamically parameterizes R_{day} ([Equation 5](#)), while Model II integrates photorespiration into an apparent rate (R_{pa} ; [Equation 14](#)), effectively decoupling photorespiratory flux from predefined biochemical ratios. This innovation enables direct empirical estimation of R_{pa0} from gas exchange data, especially under hypoxic conditions (e.g., 2% O₂) where photorespiration suppression exposes the FvCB model's limitations. Specifically, the fixed $R_{\text{day}}/V_{\text{cmax}}$ assumption the FvCB model ([von Caemmerer, 2013](#)) leads to 46%-47% underestimation of respiratory activity compared to experimental data ([Tables 1, 2](#)), whereas the new models' dynamic parameterization achieves precise alignment with observations.

Methodologically, the FvCB model's indirect estimation of $J_{A_{\text{max}}}$ via A_n - C_i curve fitting propagates errors from uncertain electron transport partitioning ([Sharkey et al., 2007](#)). Models I/II circumvent this limitation through direct quantification of A_{max} , Γ , C_{TPU} , and R_{pa0} , eliminating error accumulation inherent to multi-step biochemical approximations. This distinction explains their

robust performance across 2% and 21% O₂ environments (Figures 3, 4), demonstrating adaptability to oxygen fluctuations in both agricultural and natural ecosystems.

The FvCB model's systematic underestimation of V_{cmax} and R_{day} during A_n - C_a curve fitting further highlights its sensitivity to stomatal conductance dynamics—a confounding factor absent in A_n - C_i analyses. Models I/II address this through unified C_a - C_i treatment within a single analytical framework (Equations 5, 12), minimizing stomatal-induced artifacts. This proves critical under 2% O₂, where stomatal closure amplifies discrepancies in FvCB-derived parameters. Additionally, the non-asymptotic formulation of Models I/II (Equations 5, 12) better captures C_{TPU} inflection points than the FvCB model's segmented approach (Figures 3, 4), which struggles to resolve RuBP- versus TPU-limited transitions under dynamic conditions.

However, these advancements come with trade-offs: Models I/II cannot estimate V_{cmax} or $J_{A-\text{max}}$ —parameters critical for Rubisco kinetic analyses (Farquhar et al., 1980; Bernacchi et al., 2013). This reflects the inherent tension between empirical accuracy and biochemical interpretability, necessitating context-specific model selection. Future hybrid frameworks could integrate Model I/II's empirical strengths with the FvCB model's biochemical resolution, particularly to disentangle photorespiratory and respiratory fluxes—a capability demonstrated in low-O₂ environments where traditional partitioning assumptions fail (Xiong et al., 2022).

Enhanced predictive accuracy comes with increased parameterization complexity. Determining nuanced parameters like R_{day} requires high-precision gas exchange measurements and measured method under controlled conditions (Medlyn et al., 2011; Yin and Amthor, 2024), posing challenges for resource-limited studies. Furthermore, while coefficients α_c/α_{c1} , β_c/β_{c1} and γ/γ_{c1} in Models I/II are environment-dependent (Equations 5, 12), their biochemical basis—particularly regarding Rubisco carboxylation-oxygenation kinetics—remains unresolved. Clarifying these relationships through multi-omics approaches (e.g., concurrent chlorophyll fluorescence and metabolomic profiling) could bridge empirical models with photosynthetic biochemistry (Smith et al., 2023), transforming them into mechanistically robust tools for climate resilience research.

Furthermore, the limited sample size in our study may compromise the generalizability of our findings. Our experiments focused exclusively on two C₃ plant species, *L. perenne* and *T. aestivum*. As such, additional validation is essential to establish the broader applicability of the new models to other plant species, particularly those with distinct photosynthetic pathways, such as C₄ and CAM plants. Moreover, the models were evaluated under specific environmental conditions (2% and 21% O₂ concentrations), and their performance under other stressors—such as high temperature, drought, or elevated CO₂ levels—has yet to be fully explored. Additionally, to enhance the accuracy of R_{pa0} estimation, we recommend incorporating several additional measurement points at low CO₂ concentrations (below 200 $\mu\text{mol}\cdot\text{mol}^{-1}$), specifically at 30, 80, and 150 $\mu\text{mol}\cdot\text{mol}^{-1}$. This refinement, however, necessitates increased time and effort to obtain comprehensive A_n - C_a or A_n - C_i curves.

5 Conclusions

In conclusion, this study underscores the essential requirement for accurate model parameters and their relevance when selecting photosynthesis models. It also highlights the significance of ongoing efforts to enhance these models to improve predictions of plant photosynthesis under diverse environmental conditions. Further exploration into the molecular mechanisms underlying Rubisco-catalyzed reactions and photorespiration is crucial, as it will not only refine model accuracy but also bolster our predictive capabilities in the face of environmental changes. Such advancements are pivotal to optimizing agricultural strategies and ecological preservation.

Moreover, both Model I and Model II have shown remarkable performance, particularly under varying oxygen levels, positioning them as valuable tools for analyzing C₃ plant photosynthesis. Their consistent and reliable estimation of key parameters such as A_{max} , Γ , and R_{pa0} , coupled with their proficiency in fitting both A_n - C_a and A_n - C_i curves, offers a more precise depiction of plant photosynthetic mechanisms. As research progresses, future research should focus on validating these models across a broader range of temperatures, light intensities, and CO₂ levels to enhance their robustness. Additionally, adapting these models for C₄ and CAM plants by incorporating their unique biochemical pathways, such as PEPCase activity in C₄ plants and temporal CO₂ uptake in CAM plants, would significantly expand their applicability. Extensive validation across multiple species and conditions is essential to refine the models and ensure their accuracy. Comparative studies across different photosynthetic pathways will highlight areas for improvement and contribute to the development of generalized frameworks applicable across diverse plant types. Integrating these models into ecosystem models could also provide valuable insights into carbon cycling and ecosystem dynamics. Overall, although the new models are promising, more adaptation and validation are required to fully tap their potential in predicting photosynthetic responses among different plant species under various environmental conditions.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Author contributions

ZY: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Methodology, Writing – original draft. XY: Data curation, Investigation, Methodology, Visualization, Writing – original draft. ZY: Data curation, Investigation, Methodology, Visualization, Writing – original draft. TA: Data curation, Investigation, Methodology, Writing – original draft. SD: Data

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2025.1575217/full#supplementary-material>

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