High mesophyll conductance in the high-yielding rice cultivar Takanari quantified with the combined gas exchange and chlorophyll fluorescence measurements under free-air CO$_2$ enrichment

Hiroki Ikawa$^{a}$, Hidemitsu Sakai$^{a}$, Charles P. Chen$^{b}$, Tik Hang Soong$^{b}$, Seiichiro Yonemura$^{a}$, Yojiro Taniguchi$^{c}$, Mayumi Yoshimoto$^{a}$, Takeshi Tokida$^{d, e}$, Guoyou Zhang$^{a, f}$, Tsuneo Kuwagata$^{a}$, Hirofumi Nakamura$^{a}$, Mayumi Yoshimoto$^{a}$, Takeshi Tokida$^{d, e}$, Guoyou Zhang$^{a, f}$, Tsuneo Kuwagata$^{a}$, Hirofumi Nakamura$^{a}$, Tom Avenson$^{e}$ and Toshihiro Hasegawa$^{a, f}$

*Division of Climate Change, Institute for Agro-Environmental Sciences, National Agriculture and Food Research Organization, Tsukuba, Ibaraki, Japan; $^{b}$Department of Biology and Chemistry, Azusa Pacific University, Azusa, CA, USA; $^{c}$Division of Applied Genetics, Institute of Agrobiological Sciences, National Agriculture and Food Research Organization, Tsukuba, Ibaraki, Japan; $^{d}$Division of Biogeochemical Cycles, Institute for Agro-Environmental Sciences, National Agriculture and Food Research Organization, Tsukuba, Ibaraki, Japan; $^{e}$Taiyo Keiki Co. Ltd., Toda, Saitama, Japan; $^{f}$Division of Agro-Environmental Research, Tohoku Agricultural Research Center, National Agriculture and Food Research Organization, Morioka, Japan

**ABSTRACT**

An effective strategy for increasing crop production is increasing the rate of photosynthesis. In this study, we conducted gas exchange and chlorophyll fluorescence measurements for a high-yielding rice cultivar, Takanari, to identify the leaf physiological properties that contribute to high capacity for photosynthesis of the uppermost leaves before (panicle initiation stage) and after heading (grain-filling stage) in the Tsukuba free-air CO$_2$ enrichment (FACE) facility. The higher photosynthesis rate of Takanari compared with that of the commonly cultivated cultivar, Koshihikari, was mainly attributed to the greater stomatal conductance for CO$_2$ ($g_{sc}$) at the panicle initiation stage and to the greater mesophyll conductance ($g_m$) at the grain-filling stage in both current and elevated atmospheric CO$_2$ concentrations [CO$_2$]. Takanari had a higher level of leaf nitrogen content ($N_l$) compared with Koshihikari at the grain-filling stage, which led to greater $g_m$ and maximum carboxylation rate ($V_{c,max}$), but $N_l$ alone did not explain the variations of $g_m$ within the variety. A clear correlation was found between $V_{c,max}$ and $N_l$. Calculating $V_{c,max}$ taking $g_m$ into consideration removed the artifact of $V_{c,max25}$ in relation to $N_l$ that was observed when $g_m$ was assumed to be infinite. Our results emphasize the need to separate the roles of $V_{c,max}$ and $g_m$ to accurately understand the ecophysiological processes that control leaf photosynthesis in Takanari.

**ARTICLE HISTORY**

Received 12 September 2018
Revised 15 March 2019
Accepted 22 May 2019

**KEYWORDS**

Takanari; free-air CO$_2$ enrichment; photosynthesis; maximum carboxylation rate; mesophyll conductance; stomatal conductance; leaf nitrogen content

**Introduction**

In the face of the world’s growing population, global crop production needs to be increased. Increasing the rate of photosynthesis is one strategy to increase crop production (Hubbart, Peng, Horton, Chen & Murchie, 2007; Long, Zhu, Naidu & Ort, 2006; Mann, 1999;...
Murchie, Pinto & Horton, 2009; Ort et al., 2015). Investigations have been conducted to understand the ecophysiological traits of available crop cultivars that support high rates of photosynthesis in order to maximize the use of available genetic resources.

High mesophyll conductance ($g_m$) supports a high rate of leaf photosynthesis. However, due to the difficulty of accurately quantifying $g_m$, the effect of mesophyll conductance is often neglected in terrestrial ecosystem models that investigate crop photosynthesis at a canopy scale (Ikawa et al., 2018). Researchers often use maximum carboxylation rate ($V_{c,\text{max}}$) based on intercellular CO$_2$ concentration ($C_c$) instead of on actual CO$_2$ concentration at the carboxylation site ($C_i$), assuming that $g_m$ is infinite and, therefore, $C_c$ is equal to $C_i$. Such an assumption leads to an underestimation of $V_{c,\text{max}}$ (apparent $V_{c,\text{max}}$) and overestimation of $C_c$. However, a growing number of studies have suggested that the impact of $g_m$ on photosynthesis is not trivial, highlighting the need to disentangle the roles of $V_{c,\text{max}}$ and $g_m$ in photosynthesis (Adachi et al., 2013; Knauer et al., 2019; Lauteri, Haworth, Serraj, Monteverdi & Centritto, 2014). Furthermore, Sun et al. (2014) demonstrated that photosynthesis models based on the apparent $V_{c,\text{max}}$ assuming infinite $g_m$ are accurate only within the limited conditions in which the $A$-$C_i$ curve was observed.

Takanari (Oryza sativa L. cv. Takanari) is known for its high grain yield (Imbe et al., 2004). The Tsukuba rice free-air CO$_2$ enrichment (FACE) experiment revealed that both high sink (i.e. large panicles) and source (i.e. high carbon supply) capacities of Takanari (Chen et al., 2014; Ikawa et al., 2018; Nakano et al., 2017) contribute to the greater grain yield with less loss in the quality in elevated atmospheric CO$_2$ concentration [CO$_2$] compared to a commonly grown cultivar, Koshihikari (Hasegawa et al., 2013, 2019; Zhang et al., 2013, 2015). Based on a canopy scale model, Ikawa et al. (2018) attributed Takanari’s high canopy photosynthesis to greater stomatal conductance ($g_{sc}$ for CO$_2$) and a better nitrogen allocation compared to Koshihikari. However, their model did not explicitly consider the effect of $g_m$. Chen et al. (2014) reported that the high leaf photosynthesis of Takanari was supported mainly by high $g_m$ in later growth stage. However, the method to quantify $g_m$ used in Chen et al. (2014) depends on the curve-fitting method, which is not necessarily recommendable if any alternative method is available (Pons et al., 2009).

Recent advancements in gas exchange measurement systems enable a wide range of users to quantify $g_m$ more reliably and easily. Using a newly-released photosynthesis system (LI-6800, LICOR, USA), this study aims to redefine the physiological traits of Takanari and determine why Takanari has a high rate of leaf photosynthesis. We set three hypotheses: (1) Takanari has greater $V_{c,\text{max}}$, $g_m$, and $g_{sc}$ than Koshihikari both in current and elevated [CO$_2$]; (2) the difference in $g_m$ between [CO$_2$] treatment and variety is greater than that of either $V_{c,\text{max}}$ or $g_{sc}$ with respect to their effects on leaf photosynthesis; and (3) Takanari’s high $V_{c,\text{max}}$ and $g_m$ are explained by high leaf nitrogen contents. The rationale for the first two hypotheses is based on the results of the study by Chen et al. (2014). The third hypothesis is based on the fact that the uppermost leaves of Takanari have a high nitrogen content (Taylaran, Adachi, Ookawa, Usuda & Hirasa, 2011), and also on recent studies reporting that the $V_{c,\text{max}}$ and $g_m$ of rice leaves are correlated to leaf nitrogen content (Cai et al., 2018).

To test these hypotheses, the objectives of this study are: (1) to calculate the $V_{c,\text{max}}$, $g_m$, and $g_{sc}$ using combined gas exchange and chlorophyll

Table 1. Measurement date, time, block, measurement order, and meteorological conditions during the combined gas exchange and fluorescence measurements in 2017. The order of the measurements was randomized for each variety (KH: Koshihikari and TN: Takanari) and for CO$_2$ treatment (A-CO$_2$: a control plot with current [CO$_2$] $\sim$390 µmol mol$^{-1}$) and E-CO$_2$: elevated [CO$_2$] $\sim$590 µmol mol$^{-1}$). Air temperature ($T_a$) and relative humidity (RH) were measured at 2 m above the ground and wind speed ($U$) was measured at 2.5 m above the ground. PPFD (photosynthetic photon flux density) is the value on the horizontal plane.

| Date   | Panicle initiation stage | Time | Block | Measurement order | $T_a$ (°C) | RH (%) | $U$ (m/s) | PPFD (µmol m$^{-2}$ s$^{-1}$) |
|--------|--------------------------|------|-------|-------------------|------------|--------|-----------|-------------------------------|
| 7/11   | 9:31–12:07               | 1    | KH (A-CO$_2$) → TN (A-CO$_2$) → KH (E-CO$_2$) → TN (E-CO$_2$) | 30.0       | 64     | 3.1       | 1638                          |
| 7/13   | 9:20–11:50               | 2    | TN (A-CO$_2$) → KH (A-CO$_2$) → TN (E-CO$_2$) → KH (E-CO$_2$) | 30.8       | 69     | 3.3       | 1610                          |
| 7/14   | 9:04–11:20               | 3    | KH (E-CO$_2$) → TN (E-CO$_2$) → KH (A-CO$_2$) → TN (A-CO$_2$) | 30.4       | 66     | 2.3       | 1562                          |
| 7/15   | 9:33–13:25               | 4    | TN (E-CO$_2$) → KH (E-CO$_2$) → TN (A-CO$_2$) → KH (A-CO$_2$) | 30.9       | 64     | 1.0       | 1551                          |
|        | Grain-filling stage      |      |       |                   |            |        |           |                               |
| 8/13   | 8:06–10:20               | 1    | KH (A-CO$_2$) → TN (A-CO$_2$) → KH (E-CO$_2$) → TN (E-CO$_2$) | 27.0       | 80     | 0.8       | 896                           |
| 8/18   | 8:12–10:43               | 2    | TN (A-CO$_2$) → KH (A-CO$_2$) → TN (E-CO$_2$) → KH (E-CO$_2$) | 27.1       | 82     | 0.6       | 1051                          |
| 8/20   | 7:36–10:30               | 3    | KH (E-CO$_2$) → TN (E-CO$_2$) → KH (A-CO$_2$) → TN (A-CO$_2$) | 25.6       | 83     | 0.3       | 700                            |
| 8/22   | 8:22–10:28               | 4    | TN (A-CO$_2$) → KH (A-CO$_2$) → TN (E-CO$_2$) → KH (E-CO$_2$) | 28.6       | 77     | 0.8       | 956                            |
| 8/24   | 7:55–10:27               | 1    | KH (A-CO$_2$) → TN (A-CO$_2$) → KH (E-CO$_2$) → TN (E-CO$_2$) | 29.0       | 71     | 0.6       | 938                            |
| 8/25   | 7:46–10:07               | 2    | TN (A-CO$_2$) → KH (A-CO$_2$) → TN (E-CO$_2$) → KH (E-CO$_2$) | 30.1       | 72     | 0.5       | 744                            |
fluorescence measurements for Koshihikari and Takanari both in current and elevated [CO$_2$]; (2) to conduct a sensitivity analysis of each parameter using the Farquhar, von Caemmerer, and Berry leaf photosynthesis model (Farquhar, von Caemmerer & Berry, 1980); and (3) to measure leaf nitrogen content and compare it with $V_{c,max}$ and $g_m$.

**Materials and methods**

**Site descriptions**

Leaf-level gas exchange measurements were conducted in the Tsukuba FACE experimental facility in Tsukubamirai, Ibaraki prefecture, Japan (35° 58’ N, 139° 60’ E, 10 m.a.s.l.). The experimental facility includes four FACE rings (hereafter referred to as E-CO$_2$ plots), with a diameter of 17 m and four control blocks (A-CO$_2$ plots) (Nakamura et al., 2012). Rice seedlings of both Koshihikari and Takanari were transplanted in experimental plots on May 24–25 in 2017. Heading occurred about 70 and 75 days after the transplanting for Koshihikari and Takanari, respectively (Hasegawa et al., 2019). Measurements were made at panicle initiation (July 11–15) stage and at grain-filling stage (August 13–25) in 2017 (Table 1).

**Combined gas exchange and chlorophyll fluorescence measurements**

The uppermost expanded leaves were targeted for gas exchange and chlorophyll fluorescence measurements with the LI-6800. The heading date varied among plants, particularly in Takanari, and flag leaves with a fully-emerged panicle were selected at the grain-filling stage. Leaf samples were exposed to photosynthetic photon flux density (PPFD) at 1,500 μmol m$^{-2}$ s$^{-1}$ for 15–20 min until the steady-state condition was achieved under [CO$_2$] of 390 μmol mol$^{-1}$ in A-CO$_2$ and 590 μmol mol$^{-1}$ in E-CO$_2$. When it was overcast, samples were first exposed to PPFD at 1,000 μmol m$^{-2}$ s$^{-1}$ for several minutes and PPFD was increased to 1,500 μmol m$^{-2}$ s$^{-1}$. The fractions of red and blue lights were set at 0.9 and 0.1, respectively. Gas exchange data including net leaf photosynthesis rate ($A_n$) and $g_{sc}$ were then logged at the steady-state condition. Once the steady-state condition was achieved, [CO$_2$] in the reference cell was decreased and was kept at 200 μmol mol$^{-1}$ for several minutes. Subsequently, [CO$_2$] was further decreased to 150, 100, 55, and 20 μmol mol$^{-1}$, and data were logged within 150 s at each CO$_2$ level. The chamber temperature and humidity were set at 30°C–35°C and 60%, respectively. Other chamber conditions were: fan speed, 10,000 rpm; flow rate to the sample cell, 500 μmol air s$^{-1}$; and over-pressure, 0.2 kPa.

Chlorophyll fluorescence measurements were conducted at the same time as the gas exchange measurements with a multiphase flash fluorometer. Genty, Briantais and Baker (1989) first reported that the electron transport rate ($J$) based on the fluorometry ($J_F$) was linearly related to the quantum yield of photosystem II. On the basis of their theory, $J_F$ was estimated as follows:

$$J_F = a_i\beta Q(F_m' - F_s)/F_m$$  \hspace{1cm} (1)

where $Q$ is PPFD and $F_m'$ and $F_s$ are maximum and steady-state chlorophyll fluorescence under illumination, respectively. We assumed that leaf light absorptance ($a_i = 0.843$) and the fraction of electron distributed to photosystem II ($\beta = 0.5$) are constant.

The multiphase flash (MPF) method was used to estimate $F_m'$ at an infinitely high saturation light (Loriaux et al., 2013). A saturation light of 8,000 μmol m$^{-2}$ s$^{-1}$ with 25% attenuation for 0.3 s was used at each of the three phases. The reasonability of the saturation light was examined in the fluorescence data during the MPF measurements (0.9 s). For measurements with low CO$_2$, the fluorescence of Koshihikari seemed to decline continuously during the third phase of MPF, but we considered that the impact on $J_F$ was minimal (Figure S1 in Supplementary Material). The MPF method increased $J_F$ by 20% (Figure S1 in Supplementary Material), and both $J$ and $J_F$ were nearly identical when measured in the laboratory under saturated light levels (data not shown). We therefore assumed actual $J$ equals $J_F$ based on our protocol at least under high PPFD conditions.

**Calculation of $V_{c,max}$ and $g_m$**

The variable $J$ method was used for calculating $g_m$ from the gas exchange and chlorophyll fluorescence measurements (Harley, Loreto, Di Marco & Sharkey, 1992) using the following equation:

$$g_m = A_n/(C_i - \{\Gamma^*J + 8(A_n + R_d)/J - 4(A_n + R_d)\})$$ \hspace{1cm} (2)

where $\Gamma^*$ is CO$_2$ compensation point at $C_i$ and $R_d$ is daytime mitochondrial respiration, which was determined to be half of the dark respiration rate for Takanari and Koshihikari measured in both A-CO$_2$ and E-CO$_2$ in the study by Noguchi et al. (2018). The calculation of $g_m$ based on the variable $J$ method is sensitive to $\Gamma^*$ and $R_d$ (Centritto, Lauteri, Monteverdi & Serraj, 2009; Pons et al., 2009). We independently performed the so-called Laisk’s method for plant
samples within the experimental plot between the two stages (August 4–7) for Koshihikari and Takanari and found that CO₂ compensation point based on Cᵢ (Cᵢ*) did not statistically differ between varieties (n = 7). Adachi et al. (2013) also reported that Γ* did not differ between rice varieties. Instead of estimating Γ* from measured Cᵢ*, we decided to estimate Γ* following Bernacchi, Portis, Nakano, von Caemmerer and Long (2002) because of the following reasons: The temperature function of gₘ used in this study (Scafaro, von Caemmerer, Evans & Atwell, 2011) is also based on Bernacchi et al. (2002) and the amount of our data was too limited to identify a temperature response of Γ*.

Our pilot measurements in the laboratory for rice plants under saturated light levels showed that the values of gₘ estimated by our protocol generally agreed with the online isotope method described in Evans and von Caemmerer (2013) using the isotope measurement system employed in Nishida, Kodama, Yonemura and Hanba (2015) (gₘ by variable J = 0.99 gₘ by online isotope) +0.02, R² = 0.66, n = 34). The good comparison was achieved when Γ* for ambient O₂ concentration ([O₂] = 21%) was tuned at 30 µmol mol⁻¹, which is relatively lower than the values estimated from the temperature function. Appropriate parameterization for Γ* awaits further investigation.

Maximum carboxylation rate (Vₖₚₑₗₐₜ) was calculated by fitting the FvCB model (Farquhar et al., 1980) to Aₙ and Cₜ for [CO₂] ranging from 20–200 µmol mol⁻¹ using the lscurvefit function of MATLAB (MATLAB R2015b, MathWorks, USA). CO₂ concentration at the carboxylation site (Cᵢ) was estimated as follows:

\[
Cᵢ = Cₜ - Aₙ/gₘ
\]  

Michaels constant for CO₂ and O₂ (Kᵢ and Kₒ, respectively) required for the calculation of Vₖₚₑₗₐₜ were based on Bernacchi et al. (2002). The values of Vₖₚₑₗₐₜ and gₘ at 25°C (Vₖₚₑₗₐₜ₃₂⁰ and gₘ₃₂⁰) were estimated based on the temperature function, as described in Bernacchi, Pimentel and Long (2003) and Scafaro et al. (2011), respectively.

We used the same unit for both gₛₑ and gₘ to compare their magnitude, although the physically more appropriate unit of gₘ is molar flux per pressure because gₘ in liquid phase is defined as the ratio of a net photosynthesis rate and the gradient of partial pressure of CO₂ (Sharkey, Bernacchi, Farquhar & Singsaas, 2007). The LI-6800 generally outputs stomatal conductance for H₂O (gₛₑ), and gₛₑ was assumed to be 1.6 times greater than gₛₑ.

### Leaf nitrogen content measurements

Leaf samples used for gas exchange and chlorophyll fluorescence measurements were sampled and dried at 80°C. Specific leaf nitrogen concentration (Nₛₑ) was calculated from nitrogen concentrations measured using an NC analyzer (Sumigraph NC-22, SCAS Ltd., Japan) and the weight of the dry samples.

### Effects of leaf parameters on RuBP-saturated photosynthesis rate

To quantitatively compare the effects of each leaf parameter (Vₖₚₑₗₐₜ, gₘ, and gₛₑ) on leaf photosynthesis, the rate of RuBP-saturated photosynthesis (Aₑ) (Farquhar et al., 1980) as a proxy of leaf photosynthesis was calculated by changing one of the parameters under the same [CO₂] of 390 µmol mol⁻¹ and in similar environmental conditions as when the measurements were taken (air and leaf temperature: 30°C; relative humidity: 60%; PPFD: 1,500 µmol m⁻² s⁻¹; and leaf boundary conductance for H₂O (gₛₑ): 3 mol m⁻² s⁻¹). The detailed calculation procedure is explained in Appendix A.

### 2016 field season data

To supplement our results with information from another field season, A-Cᵢ curves obtained at the grain-filling stage in 2016 and that were used in the study by Ikawa et al. (2018) were analyzed to obtain Vₖₚₑₗₐₜ and gₘ based on the curve-fitting method (Sharkey et al., 2007). Note that Ikawa et al. (2018) in their study calculated apparent Vₖₚₑₗₐₜ neglecting gₘ. The Excel tool provided in the study by Sharkey et al. (2007) was modified so that respiration rate was fixed at the same rate as that of the grain-filling stage data in 2017, and the temperature function for gₘ of Oryza sativa reported in Scafaro et al. (2011) was introduced. Further details about the measurements can be found in the study by Ikawa et al. (2018).

### Statistical analysis

A split-plot approach was used with variety effect nested within [CO₂] and replicate blocks (n = 4). An R script used in the study by Maurit et al. (2017) and Ikawa et al. (2018) was adapted for the analysis. Measurements were taken twice at two of the blocks on different days at the grain-filling stage, and the data were treated as subsets of each block. The overall variation of the simulated Aₑ was smaller than the variation
of other parameters, and the variety effect and CO₂ effect were tested separately.

Results

Takanari had a higher $A_n$ than Koshihikari under both CO₂ treatments and in both panicle initiating and grain-filling stages (Figure 1(a,b) and Table 2). E-CO₂ increased $A_n$ for both varieties at both growth stages, but the increase for Koshihikari was not statistically significant at the grain-filling stage. At the grain-filling stage, the $A_n$ of Takanari responded to CO₂ more strongly (30%) than did that of Koshihikari (2%), and the effect of CO₂ treatment and variety difference in $A_n$ was significant.

There was not a clear varietal difference in $V_{c,max}$ at the panicle initiation stage, but Takanari had a
greater $V_{c,\text{max}25}$ than did Koshihikari at the grain-filling stage, both in 2016 and 2017 (Figure 1(c,d) and Table 2). The downregulation of $V_{c,\text{max}25}$ under E-CO$_2$ was also evident at the grain-filling stage, although in 2016 it was not statistically significant. The varietal differences in $g_{sc}$ and $g_{m25}$ were more apparent than in the case of $V_{c,\text{max}25}$ at both panicle initiation stage and grain-filling stage (Figure 1(e,h) and Table 2). E-
CO₂ also decreased both \(g_{m25}\) and \(g_{sc}\) at the grain-filling stage. The magnitude of \(g_{sc}\) was always greater than that of \(g_{m}\), and their ratio was greater in A-CO₂ (~1.5) than it was in E-CO₂ (~1.3) at the panicle initiation stage, and greater in Koshihikari (~1.7) than in Takanari (~1.2) at the grain-filling stage.

The varietal difference in \(g_{sc}\) had a significant impact on the calculated \(A_c\) at the panicle initiation stage, and the varietal difference in \(g_{m}\) had a significant effect on \(A_c\) at the grain-filling stage (Figure 2 and Table 2). Although varietal differences in \(V_{c,\text{max}25}\) and \(g_{sc}\) were found at the grain-filling stage, they did not lead to a statistically significant difference in \(A_c\) between the varieties (Table 2).

A clear correlation \((R^2 = 0.60)\) was found between \(V_{c,\text{max}25}\) and \(N_l\), and the correlation was much clearer when \(V_{c,\text{max}25}\) was calculated taking \(g_{m}\) into consideration rather than assuming \(g_{m}\) to be infinite (i.e. apparent \(V_{c,\text{max}25}\)) (Figure 3(a,b)). Analysis of covariance (ANCOVA) indicated that the relationship between \(V_{c,\text{max}25}\) and \(N_l\) was not different between Koshihikari and Takanari, but that the relationship between apparent \(V_{c,\text{max}25}\) and \(N_l\) significantly differed between varieties \((p < 0.0005)\). Apparent \(V_{c,\text{max}25}\) of each variety was also smaller than that estimated from \(N_l\) based on the data from 2016 and on the relationship reported in Ikawa et al. (2018) (Figure 3(b)). Interestingly, the relationship between \(V_{c,\text{max}25}\) and \(N_l\) was comparable between 2016 and 2017 (Figure 3(a)), despite using different methods and instruments to estimate \(V_{c,\text{max}25}\). For the data in 2016, Ikawa et al. (2018) used a LI-6400 photosynthesis system, but we found no significant difference in the measured apparent \(V_{c,\text{max}25}\) between LI-6400 and LI-6800 (Figure S2 in Supplementary Material).

A correlation was also found between \(N_l\) and \(g_{m25}\) \((R^2 = 0.37)\), but the relationship within the variety was obscure \((R^2 = 0.20\) for Koshihikari and \(R^2 = 0.24\) for Takanari). Takanari had greater \(N_l\) than did Koshihikari at the grain-filling stage. At the panicle initiation stage, E-CO₂ also decreased \(N_l\) for both varieties, but the decrease was not statistically significant \((p = 0.1)\).

**Discussion**

Our results partly support the hypothesis that Takanari has greater \(V_{c,\text{max}}\), \(g_{m}\), and \(g_{sc}\) than does Koshihikari both in current and elevated [CO₂]. Both \(g_{m25}\) and \(g_{sc}\) were greater in Takanari than in Koshihikari, but \(V_{c,\text{max}25}\) did not differ between varieties at the panicle initiation stage. There was no clear varietal difference in \(V_{c,\text{max}25}\) at the panicle initiation stage and only a possible difference \((p = 0.06)\) was detected at the grain-filling stage. This result might be related to \(N_l\). Takanari had a greater \(N_l\) than did Koshihikari at the grain-filling stage, but \(N_l\) did not differ significantly at the panicle initiation stage (data not shown). In the same FACE experiment, Muryono et al. (2017) reported that \(N_l\) in the top canopy leaves was even lower in Takanari than

![Figure 3. Maximum carboxylation rate at 25°C (\(V_{c,\text{max}25}\)) in relation to leaf nitrogen content (\(N_l\)) (a); \(V_{c,\text{max}25}\) with mesophyll conductance \((g_{m})\) assumed to be infinite (apparent \(V_{c,\text{max}25}\)) in relation to \(N_l\) (b); \(g_m\) at 25°C \((g_{m25}\)) in relation to \(N_l\) (c). ANCOVA (analysis of covariance) indicates that the relationship between \(V_{c,\text{max}25}\) or \(g_{m25}\) and \(N_l\) was different for Koshihikari and Takanari in (b) and (c). No difference was found between CO₂ treatments (i.e. A-CO₂ and E-CO₂).](image-url)
it was in Koshihikari at the panicle initiation stage. Generally, Takanari begins to show a high level of $N_t$ in the top canopy leaves after heading (e.g. Taylaran et al., 2011), and it is likely that the $V_{c,max}$ of Takanari also follows the seasonal trend of $N_t$.

Chen et al. (2014) reported in their study that the smaller mesophyll conductance limitation (MCL) of Takanari resulted in a higher rate of photosynthesis than that of Koshihikari, and that the varietal difference in MCL was greater than that of stomatal conductance limitation (SCL), particularly at later growth stages. Our results at the grain-filling stage agree with the results of the study by Chen et al. (2014) and support our second hypothesis: the difference in $g_m$ between [CO$_2$] treatment and variety is greater than that of either $V_{c,max}$ or $g_{sc}$ with respect to their effects on leaf photosynthesis. At the panicle initiation stage, however, our results suggested that the varietal difference in SCL was greater than it was in MCL. The small varietal difference in $g_m$ at the panicle initiation stage might be due to the small varietal difference in $N_t$ as compared with the grain-filling stage.

The third hypothesis: Takanari’s high $V_{c,max}$ and $g_m$ are explained by high leaf nitrogen contents, was also partly supported by our results. The greater $N_t$ of Takanari correlated with the greater $V_{c,max}$ and $g_{m25}$ as compared with Koshihikari at the grain-filling stage. A high $N_t$ is considered to indicate an anatomical advantage for $g_m$ by increasing the effective surface of chloroplast (Cai et al., 2018). However, the correlation between $N_t$ and $g_{m25}$ was not as strong as the correlation between $N_t$ and $V_{c,max}$, and the relation was not clear within varieties, suggesting that other factors influence $g_{m25}$. Responses to environmental changes (Flexas et al., 2007; Mizokami, Noguchi, Kojima, Sakakibara & Terashima, 2015), anatomical structure (Adachi et al., 2013; Terashima, Miyazawa & Hanba, 2001), and aquaporins that possibly control not only H$_2$O transport but also the transport of other materials including CO$_2$ (Ferrio et al., 2012; Miyazawa, Yoshimura, Shinzaki, Maeshima & Miyake, 2008) could all influence $g_m$.

Our results indicate that $g_m$ is significant for determining leaf photosynthesis; that its importance changes with the growth stage; and that its variation is not simply explained by $N_t$. Furthermore, apparent $V_{c,max}$ given the same $N_t$ was estimated to be higher in Takanari than Koshihikari, and was generally higher in 2016 measurements than in 2017 measurements (Figure 3(b)); however, such bias in $V_{c,max}$ was no longer observed when $g_m$ was considered in the calculation. These results emphasize the importance of separately considering the roles of $V_{c,max}$ and $g_m$ for accurately understanding the ecophysiological processes that control leaf photosynthesis.

The magnitudes of $V_{c,max}$ and $g_{m25}$ and their relation to $N_t$ were comparable between 2016 and 2017, even though different instruments (LI-6400 in 2016 and LI-6800 in 2017) and different methods (curve-fitting method in 2016 and variable $J$ method in 2017) were used (Figures 1 and 3). When the values of $g_m$ reported in the study by Chen et al. (2014) were converted to 25°C according to the temperature function of Scafaro et al. (2011), they ranged roughly from 0.1 to 0.15 mol m$^{-2}$ s$^{-1}$ for Koshihikari and 0.15 to 0.2 mol m$^{-2}$ s$^{-1}$ for Takanari in the mid grain-filling stage in 2012 and the heading stage in 2013. These values are comparable to those of our present study (Figure 1). Our results suggest that the curve-fitting method would be useful to recompute $V_{c,max}$ and $g_m$ based on $A$-$C$$_{i}$ curves obtained in the past as long as careful comparisons between the measurement techniques are made.

Elevated CO$_2$ decreased $V_{c,max}$, $g_m$, and $g_{sc}$ at the grain-filling stage. Early studies have reported that higher CO$_2$ decreases $g_m$ (e.g. Flexas et al., 2007; Yin et al., 2009), but the validity of the short-term response of $g_m$ to CO$_2$ is debatable, partly because the calculation of $g_m$ requires $C_l$ as an input (Gu & Sun, 2014; Tazoe, von Caemmerer, Badger & Evans, 2009). To examine whether the CO$_2$ response was the artefact, Mizokami, Noguchi, Kojima, Sakakibara and Terashima (2019) demonstrated that Arabidopsis thaliana with impaired stomatal regulation decreased $g_m$ with increasing CO$_2$, which supports a hypothesis that the short-term response of $g_m$ to CO$_2$ does occur. It is also known that $g_m$ decreases during long-term exposure to high CO$_2$ (Cai et al., 2018; Mizokami et al., 2019). To investigate whether the decrease of $g_m$ in E-CO$_2$ in the grain-filling stage was due to the direct response to CO$_2$ or to acclimation effects, we calculated $g_{m25}$ when [CO$_2$] of the reference cell was set at 150 μmol mol$^{-1}$. The paired $t$-test found no statistically significant difference in $g_{m25}$ between the growth CO$_2$ condition and [CO$_2$] at 150 μmol mol$^{-1}$. However, $g_{m25}$ was still lower in the plants grown in E-CO$_2$ than in those grown in A-CO$_2$ under the same [CO$_2$] of 150 μmol mol$^{-1}$ ($p < 0.05$). We therefore consider that the decrease of $g_m$ in E-CO$_2$ was due to acclimation rather than to a short-term response to CO$_2$.

The effects of decreased $V_{c,max}$, $g_m$, and $g_{sc}$ in E-CO$_2$ on $A_t$ were small and not statistically significant. Cai et al. (2018) also reported that the CO$_2$ acclimation effect on photosynthesis was small in rice leaves. It should be noted, however, that E-CO$_2$ increased $A_t$ of Takanari much more than that of Koshihikari at the grain-filling
stage (Figure 1(b) and Table 2), even though a variety×CO₂ interaction effect was not found in any of the single parameter (Table 2). This implies the combined effects of CO₂ acclimation of physiological parameters were greater in Koshihikari than in Takanari at the grain-filling stage. If CO₂ acclimation did not occur and Vₚ, max, gₘ, and gₛₑ were assumed to be invariant between CO₂ treatments, then further sensitivity analysis with the FvCB model showed that E-CO₂ benefits Aₚ of Koshihikari slightly more than that of Takanari, which agrees with the theory that high CO₂ benefits individuals with lower conductance (Centritto, Tognetti, Leitgeb, Štrélcová & Cohen, 2011).

We consider that the relevance of this study to crop production is limited by the fact that we conducted gas exchange and chlorophyll fluorescence measurements under a similar environment with a high level of PPFD. The performance of the MPF method under different light conditions has yet to be investigated. While rice physiology rapidly changes through the growth (Tatsumi, Kuwabara & Motobayashi, 2019), it was not possible to completely eliminate potential effects of different phenology between Koshihikari and Takanari. Another limitation of our study is that we did not consider the interaction between physiological effects and plants’ energy balance (Ikawa et al., 2018; Yoshimoto, Oue & Kobayashi, 2005) that impact the thermal environment and thus photosynthesis and rice production (Usui et al., 2014, 2016).

Conclusions

Vₚ, max, gₘ, and gₛₑ of the uppermost leaves of the high-yielding rice cultivar Takanari were quantified and compared with those in Koshihikari under current and elevated [CO₂] before and after heading. The effects of the differences in these physiological parameters between varieties and CO₂ treatments on Aₚ were then quantified, using Aₚ as a proxy of leaf photosynthesis rate. The greater Aₚ of Takanari than of Koshihikari was mainly supported by high gₛₑ at the panicle initiation stage and by high gₘ at the grain-filling stage. Calculating Vₚ, max, in relation to Nₑ that was observed when gₘ was assumed to be infinite. Although Nₑ may provide a good estimate of Vₚ, max, other factors besides Nₑ play a role in the variations of gₘ. Our results highlight the importance of considering gₘ to accurately understand photosynthetic processes and the need to further explore the mechanisms regulating gₘ. E-CO₂ decreased all three parameters (Vₚ, max, gₘ, and gₛₑ) at the grain-filling stage, and the decrease in gₘ is likely to be due to acclimation to high CO₂.

Acknowledgments

This study was supported by JSPS KAKENHI 17H03896, 19H03077, and 16H06279 (PAGS). The authors thank Ms. Mariko Marushima and Ms. Yoshiko Higashikozono for the NC analysis and Mr. Yuta Inoue (Meiwafosis) for a technical support for the LI-6400 and LI-6800.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the JSPS KAKENHI [grant number 17H03896]; JSPS KAKENHI [grant number 16H06279]; and JSPS KAKENHI [grant number 19H03077].

ORCID

Hiroki Ikawa http://orcid.org/0000-0002-4984-8067
Takeshi Tokida http://orcid.org/0000-0001-7245-2952
Toshihiro Hasegawa http://orcid.org/0000-0001-8501-5612

References

Adachi, S., Nakae, T., Uchida, M., Soda, K., Takai, T., Oi, T., … Hirasawa, T. (2013). The mesophyll anatomy enhancing CO₂ diffusion is a key trait for improving rice photosynthesis. _Journal of Experimental Botany_, 64(4), 1061–1072.

Bernacchi, C. J., Pimentel, C., & Long, S. P. (2003). In vivo temperature response functions of parameters required to model RuBP-limited photosynthesis. _Plant, Cell & Environment_, 26(9), 1419–1430.

Bernacchi, C. J., Portis, A. R., Nakano, H., von Caemmerer, S., & Long, S. P. (2002). Temperature response of mesophyll conductance. Implications for the determination of rubisco enzyme kinetics and for limitations to photosynthesis in vivo. _Plant Physiology_, 130(4), 1992–1998.

Cai, C., Li, G., Yang, H., Yang, J., Liu, H., Struijk, P. C., … Zhu, J. (2018). Do all leaf photosynthesis parameters of rice acclimate to elevated CO₂, elevated temperature, and their combination, in FACE environments? _Global Change Biology_, 24(4), 1685–1707.

Centritto, M., Lauteri, M., Monteverdi, M. C., & Serraj, R. (2009). Leaf gas exchange, carbon isotope discrimination, and grain yield in contrasting rice genotypes subjected to water deficits during the reproductive stage. _Journal of Experimental Botany_, 60(8), 2325–2339.

Centritto, M., Tognetti, R., Leitgeb, E., Štrélcová, K., & Cohen, S. (2011). Above ground processes: Anticipating climate change influences. In M. Bredemeier, S. Cohen, D. L. Godbold, E. Lode, V. Pichler, & P. Schleppi (Eds.), _Forest management and the water cycle: An ecosystem-based approach_ (pp. 31–64). doi:10.1007/978-3-642-9834-1_3

Chen, C. P., Sakai, H., Tokida, T., Usui, Y., Nakamura, H., & Hasegawa, T. (2014). Do the rich always become richer? Characterizing the leaf physiological response of the high-
yielding rice cultivar takanari to free-air CO₂ enrichment. *Plant and Cell Physiology*, 55(2), 381–391.

Ethier, G. J., & Livingston, N. J. (2004). On the need to incorporate sensitivity to CO₂ transfer conductance into the Farquhar-von Caemmerer-Berry leaf photosynthesis model. *Plant, Cell and Environment*, 27(2), 137–153.

Evans, J. R., & von Caemmerer, S. (2013). Temperature response of carbon isotope discrimination and mesophyll conductance in tobacco: Temperature response of mesophyll conductance. *Plant, Cell & Environment*, 36(4), 745–756.

Farquhar, G. D., von Caemmerer, S., & Berry, J. A. (1980). A biochemical model of photosynthetic CO₂ assimilation in leaves of C₃ species. *Planta*, 149(1), 78–90.

Ferrio, J. P., Pou, A., Florez-Sarasa, I., Gessler, A., Kodama, N., Flexas, J., & Ribas-Carbo, M. (2012). The Péclét effect on leaf water enrichment correlates with leaf hydraulic conductance and mesophyll conductance for CO₂. *Plant, Cell & Environment*, 35(3), 611–625.

Flexas, J., Díaz-Espejo, A., Galmés, J., Kaldenhoff, R., Medrano, H., & Ribas-Carbo, M. (2007). Rapid variations of mesophyll conductance in response to changes in CO₂ concentration around leaves. *Plant, Cell & Environment*, 30(10), 1284–1298.

Genty, B., Briantais, J.-M., & Baker, N. R. (1989). The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica Et Biophysica Acta (Bba)-General Subjects*, 990 (1), 87–92.

Gu, L., & Sun, Y. (2014). Artefactual responses of mesophyll conductance to CO₂ and irradiance estimated with the variable J and online isotope discrimination methods. *Plant, Cell & Environment*, 37(5), 1231–1249.

Harley, P. C., Loreto, F., Di Marco, G., & Sharkey, T. D. (1992). Theoretical considerations when estimating the mesophyll conductance to CO₂ flux by analysis of the response of photosynthesis to CO₂. *Plant Physiology*, 98(4), 1429–1436.

Hasegawa, T., Sakai, H., Tokida, T., Nakamura, H., Zhu, C., Usui, Y., . . . Makino, A. (2013). Rice cultivar responses to elevated CO₂ at two free-air CO₂ enrichment (FACE) sites in Japan. *Functional Plant Biology*, 40(2), 148–159.

Hasegawa, T., Sakai, H., Tokida, T., Usui, Y., Nakamura, H., Wakatsuki, H., . . . Hayashi, K. (2019). A high-yielding rice cultivar “Takanari” shows no N constraints on CO₂ fertilization. *Frontiers in Plant Science*, 10, 361.

Hubbard, S., Peng, S., Horton, P., Chen, Y., & Murchie, E. H. (2007). Trends in leaf photosynthesis in historical rice varieties developed in the Philippines since 1966. *Journal of Experimental Botany*, 58(12), 3429–3438.

Ikawa, H., Chen, C. P., Sikma, M., Yoshimoto, M., Sakai, H., Tokida, T., . . . Hasegawa, T. (2018). Increasing canopy photosynthesis in rice can be achieved without a large increase in water use-A model based on free-air CO₂ enrichment. *Global Change Biology*, 24(3), 1321–1341.

Imbe, T., Akama, Y., Nakane, A., Hata, T., Ise, K., Ando, I., . . . Koga, Y. (2004). Development of a multipurpose high-yielding rice variety “Takanari”. *Bulletin of the National Institute of Crop Science*, 5, 35–51. (in Japanese with English abstract).

Knauer, J., Zaehele, S., Kauwe, M. G. D., Bahar, N. H. A., Evans, J. R., Medlyn, B. E., . . . Werner, C. (2019). Effects of mesophyll conductance on vegetation responses to elevated CO₂ concentrations in a land surface model. *Global Change Biology*, 25(5), 1820–1838.

Lauteri, M., Haworth, M., Serraj, R., Monteverdi, M. C., & Centritto, M. (2014). Photosynthetic diffusional constraints affect yield in drought stressed rice cultivars during flowering. *Plos One*, 9(10), e109054.

Long, S. P., Zhu, X.-G., Naidu, S. L., & Ort, D. R. (2006). Can improvement in photosynthesis increase crop yields? *Plant, Cell & Environment*, 29(3), 315–330.

Loriaux, S. D., Avenson, T. J., Welles, J. M., Mcdermitt, D. K., Eckles, R. D., Riensche, B., & Genty, B. (2013). Closing in on maximum yield of chlorophyll fluorescence using a single multiphase flash of sub-saturating intensity: Fm using on single multiphase flash. *Plant, Cell & Environment*, 36(10), 1755–1770.

Mann, C. C. (1999). FUTURE FOOD: Crop scientists seek a new revolution. *Science*, 283(5400), 310–314.

Mauritz, M., Bracho, R., Celis, G., Hutchings, J., Natali, S. M., Pegoraro, E., . . . Schuur, E. A. G. (2017). Non-linear CO₂ flux response to seven years of experimentally induced permafrost thaw. *Global Change Biology*, 23(9), 3646–3666.

Miyazawa, S.-I., Yoshimura, S., Shinzaki, Y., Maeshima, M., & Miyake, C. (2008). Deactivation of aquaporins decreases internal conductance to CO₂ diffusion in tobacco leaves grown under long-term drought. *Functional Plant Biology*, 35(7), 553–564.

Mizokami, Y., Noguchi, K., Kojima, M., Sakakibara, H., & Terashima, I. (2015). Mesophyll conductance decreases in the wild type but not in an ABA-deficient mutant (aba1) of *Nicotiana plumbaginifolia* under drought conditions: Stomatal and mesophyll CO₂ conductances under drought conditions. *Plant, Cell & Environment*, 38(3), 388–398.

Mizokami, Y., Noguchi, K., Kojima, M., Sakakibara, H., & Terashima, I. (2019). Effects of instantaneous and growth CO₂ levels and abscisic acid on stomatal and mesophyll conductances. *Plant, Cell & Environment*, 42(4), 1257–1269.

Murchie, E. H., Pinto, M., & Horton, P. (2009). Agriculture and the new challenges for photosynthesis research. *New Phytologist*, 181(3), 532–552.

Muruyono, M., Chen, C. P., Sakai, H., Tokida, T., Hasegawa, T., Usui, Y., . . . Hikosaka, K. (2017). Nitrogen distribution in leaf canopies of a high-yielding rice (Oryza sativa L.) cultivar Takanari. *Crop Science*, 57(4), 2080–2088.

Nakamura, H., Tokida, T., Yoshimoto, M., Sakai, H., Fukuoka, M., & Hasegawa, T. (2012). Performance of the enlarged RiceFACE system using pure CO₂ installed in Tsukuba, Japan. *Journal of Agricultural Meteorology*, 68(1), 15–23.

Nakano, H., Yoshinaga, S., Takai, T., Arai-Sanoh, Y., Kondo, K., Yamamoto, T., . . . Kondo, M. (2017). Quantitative trait loci for large sink capacity enhance rice grain yield under free-air CO₂ enrichment conditions. *Scientific Reports*, 7(1), 1827.

Nishida, K., Kodama, N., Yonemura, S., & Hanba, Y. T. (2015). Rapid response of leaf photosynthesis in two fern species *Pteridium aquilinum* and *Thelypteris dentata* to changes in CO₂ concentration. *Journal of Agricultural Meteorology*, 68(1), 15–23.

Ort, D. R., Merchant, S. S., Alric, J., Barkan, A.,Blankenship, R. E., Bock, R., . . . Zhu, X. G. (2015). Redesigning photosynthesis
to sustainably meet global food and bioenergy demand. *Proceedings of the National Academy of Sciences*, 112(28), 8529–8536.

Pons, T. L., Flexas, J., von Caemmerer, S., Evans, J. R., Genty, B., Ribas-Carbo, M., & Brugnoli, E. (2009). Estimating mesophyll conductance to CO₂: Methodology, potential errors, and recommendations. *Journal of Experimental Botany*, 60(8), 2217–2234.

Scafaro, A. P., von Caemmerer, S., Evans, J. R., & Atwell, B. J. (2011). Temperature response of mesophyll conductance in cultivated and wild Oryza species with contrasting mesophyll cell wall thickness: Temperature response of mesophyll conductance in rice. *Plant, Cell & Environment*, 34(11), 1999–2008.

Sharkey, T. D., Bernacchi, C. J., Farquhar, G. D., & Singsaas, E. L. (2007). Fitting photosynthetic carbon dioxide response curves for C₃ leaves. *Plant, Cell & Environment*, 30(9), 1035–1040.

Sun, Y., Gu, L., Dickinson, R. E., Norby, R. J., Pallardy, S. G., & Hoffman, F. M. (2014). Impact of mesophyll diffusion on estimated global land CO₂ fertilization. *Proceedings of the National Academy of Sciences*, 111(44), 15774–15779.

Tatsumi, K., Kuwabara, Y., & Motobayashi, T. (2019). Monthly variability in the photosynthetic capacities, leaf mass areas and leaf nitrogen contents of rice (Oryza sativa L.) plants and their correlations. *Journal of Agricultural Meteorology*, 75(2), 111–119.

Taylaran, R. D., Adachi, S., Ookawa, T., Usuda, H., & Hirasawa, T. (2011). Hydraulic conductance as well as nitrogen accumulation plays a role in the higher rate of leaf photosynthesis of the most productive variety of rice in Japan. *Journal of Experimental Botany*, 62(11), 4067–4077.

Tazoe, Y., von Caemmerer, S., Badger, M. R., & Evans, J. R. (2009). Light and CO₂ do not affect the mesophyll conductance to CO₂ diffusion in wheat leaves. *Journal of Experimental Botany*, 60(8), 2291–2301.

Terashima, I., Miyazawa, S.-I., & Hanba, Y. T. (2001). Why are sun leaves thicker than shade leaves? — Consideration based on analyses of CO₂ diffusion in the leaf. *Journal of Plant Research*, 114(1), 93–105.

Usui, Y., Sakai, H., Tokida, T., Nakamura, H., Nakagawa, H., & Hasegawa, T. (2014). Heat-tolerant rice cultivars retain grain appearance quality under free-air CO₂ enrichment. *Rice*, 7(1), 6.

Usui, Y., Sakai, H., Tokida, T., Nakamura, H., Nakagawa, H., & Hasegawa, T. (2016). Rice grain yield and quality responses to free-air CO₂ enrichment combined with soil and water warming. *Global Change Biology*, 22(3), 1256–1270.

von Caemmerer, S., & Evans, J. R. (1991). Determination of the average partial pressure of CO₂ in chloroplasts from leaves of several C₃ plants. *Australian Journal of Plant Physiology*, 18(3), 287.

von Caemmerer, S., & Farquhar, G. D. (1981). Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta*, 153(4), 376–387.

Yin, X., Struijk, P. C., Romero, P., Harbinson, J., Evers, J. B., Van Der Putten, P. E. L., & Vos, J. (2009). Using combined measurements of gas exchange and chlorophyll fluorescence to estimate parameters of a biochemical C₃ photosynthesis model: A critical appraisal and a new integrated approach applied to leaves in a wheat (*Triticum aestivum*) canopy. *Plant, Cell & Environment*, 32(5), 448–464.

Yoshimoto, M., Oue, H., & Kobayashi, K. (2005). Energy balance and water use efficiency of rice canopies under free-air CO₂ enrichment. *Agricultural and Forest Meteorology*, 133(1–4), 226–246.

Zhang, G., Sakai, H., Tokida, T., Usui, Y., Zhu, C., Nakamura, H., ... Hasegawa, T. (2013). The effects of free-air CO₂ enrichment (FACE) on carbon and nitrogen accumulation in grains of rice (Oryza sativa L.). *Journal of Experimental Botany*, 64(11), 3179–3188.

Zhang, G., Sakai, H., Usui, Y., Tokida, T., Nakamura, H., Zhu, C., ... Hasegawa, T. (2015). Grain growth of different rice cultivars under elevated CO₂ concentrations affects yield and quality. *Field Crops Research*, 179, 72–80.
Appendix A. Calculation of RuBP-saturated photosynthesis rate ($A_c$)

RuBP-saturated photosynthesis rate ($A_c$) in the FvCB model (Farquhar et al., 1980) was calculated by iteratively solving Equations (A-1) and (A-2). The quadratic equation (Equation A-1) was proposed by von Caemmerer and Evans (1991) and its solution is explicitly described in Ethier and Livingston (2004)

where $C_a$ is atmospheric CO$_2$ concentration (μmol mol$^{-1}$), $C_o$ is O$_2$ concentration (μmol mol$^{-1}$). The total conductance for CO$_2$ ($g_{tc}$, mol m$^{-2}$ s$^{-1}$) and transpiration rate ($E$, mol m$^{-2}$ s$^{-1}$) are calculated as follows (von Caemmerer & Farquhar, 1981):

$$g_{tc} = \left\{ \frac{1}{g_{tc}} + 1/(1.37g_{bw}) \right\}^{-1} \quad (A-3)$$

$$E = g_{sw}(w_i - w_a)/(1 - (w_i + w_a)/2) \quad (A-4)$$

where $w_i$ and $w_a$ are H$_2$O molar fraction at the intercellular space and atmosphere, respectively (mol mol$^{-1}$). The total conductance for H$_2$O ($g_{bw}$, mol m$^{-2}$ s$^{-1}$) was defined as follows:

$$g_{bw} = \left\{ \frac{1}{g_{sw}} + 1/g_{bw} \right\}^{-1} \quad (A-5)$$

Equations (A-1)–(A-5) were coded with MATLAB (MATLAB R2015b, MathWorks, USA).