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Comparison of parameters estimated from A/C_i and A/C_c curve analysis

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Abstract

The parameters estimated from traditional A/C_i curve analysis are dependent upon some underlying assumptions that substomatal CO_2 concentration (C_i) equals the chloroplast CO_2 concentration (C_c) and the C_i value at which the A/C_i curve switches between Rubisco- and electron transport-limited portions of the curve (C_i-t) is set to a constant. However, the assumptions reduced the accuracy of parameter estimation significantly without taking the influence of C_i-t value and mesophyll conductance (g_m) on parameters into account. Based on the analysis of *Larix gmelinii*'s A/C_i curves, it showed the C_i-t value varied significantly, ranging from 24 Pa to 72 Pa and averaging 38 Pa. *t*-test demonstrated there were significant differences in parameters respectively estimated from A/C_i and A/C_c curve analysis ($p<0.01$). Compared with the maximum ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) carboxylation rate (V_{cmax}), the maximum electron transport rate (J_{max}) and J_{max}/V_{cmax} estimated from A/C_c curve analysis which considers the effects of g_m limit and simultaneously fits parameters with the whole A/C_c curve, mean V_{cmax} estimated from A/C_i curve analysis (V_{cmax}-C_i) was underestimated by 37.49%; mean J_{max} estimated from A/C_i curve analysis (J_{max}-C_i) was overestimated by 17.8% and (J_{max}-C_i)/(V_{cmax}-C_i) was overestimated by 24.2%. However, there was a significant linear relationship between V_{cmax} estimated from A/C_i curve analysis and V_{cmax} estimated from A/C_c curve analysis, so was it J_{max} ($p<0.05$).

Additional key words: A/C_i curve analysis; A/C_c curve analysis; mesophyll conductance; parameter estimation; photosynthesis.

Introduction

Since the middle of the 18th century, fossil fuel combustion, land use, and other human activities forced steady increase of greenhouse gas in atmosphere, especially the increase of CO_2 that leads to greenhouse effect (Keeling *et al.* 1989, Waston *et al.* 1990), which affects earth's energy balance, climate change, precipitation pattern and global climate change (Mitchell *et al.* 1990, Schlesinger *et al.* 1985), and consequently the terrestrial ecosystems (Bazzaz 1990, Larcher 1980, Melillo *et al.* 1993, Woodward *et al.* 1987).

Long (1991) pointed that predicting the responses of leaf photosynthesis to environmental factors was fundamental to projecting the impact of global change on the biosphere. Farquhar–von Caemmerer–Berry model of

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Abbreviations: A – photosynthesis rate; A_c – Rubisco-limited rates of carboxylation; A_j – electron transport-limited rates of carboxylation; A/C_c curve analysis – net assimilation rate of CO_2-chloroplast CO_2 concentration; A/C_i curve analysis – net assimilation rate of CO_2-intercellular CO_2 concentration; c – scaling constant; C_c – chloroplast CO_2 concentration; C_c-t – chloroplast CO_2 concentration transitional point; C_i – substomatal CO_2 concentration; C_i-t – intercellular CO_2 concentration transitional point; FvCB model – Farquhar–von–Caemmerer–Berry model of photosynthesis; g_m – mesophyll conductance; J – electron transport rate; J_{max} – maximum electron transport rate; J_{max}-C_c – J_{max} estimated from A/C_c curve analysis; J_{max}-C_i – J_{max} estimated from A/C_i curve analysis; K_c – the Michaelis-Menten constants of Rubisco activity for CO_2; K_o – the Michaelis–Menten constants of Rubisco activity for O_2; O – the O_2 partial pressure in intercellular spaces; PPFD – photosynthetic photon flux density; R – gas constant; R_D – dark respiration; V_c – the rate of carboxylation of Rubisco; V_{cmax} – maximum ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) carboxylation rate; V_{cmax}-C_c – V_{cmax} estimated from A/C_c curve analysis; V_{cmax}-C_i – V_{cmax} estimated from A/C_i curve analysis; Γ^* – the CO_2 compensation point in the absence of R_D; ΔH_a – enthalpy of activation; ΔH_d – enthalpy of deactivation; ΔS – entropy.

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photosynthesis (FvCB model) (Farquhar *et al.* 1980, Harley and Sharkey 1991, Harley *et al.* 1992ab, Sharkey 1985) due to its mechanism and generalization has been applied extensively to simulate plant photosynthesis and productivity which is a key step in calculating the carbon gains at canopy (Amthor 1995, De Pury and Farquhar 1997, Lloyd and Farquhar 1996, Wang and Jarvis 1990), ecosystem (Field and Avissar 1998), landscape (Pitman 2003, Sellers *et al.* 1996, 1997) and even global levels. The FvCB model requires several critical parameters, such as V_{cmax}, J_{max}, leaf dark respiration (R_D), and mesophyll conductance (g_m). Therefore, these major parameters are central to the prediction of plant photosynthesis capacity with the Farquhar's photosynthesis model. At present, these parameters can be calculated from the net assimilation rate of CO_2-chloroplast CO_2 concentration (A/C_c) or net assimilation rate of CO_2-intercellular CO_2 concentration (A/C_i) curve analysis.

There are two prior assumptions in parameter estimation of A/C_i curve analysis. A first one is that intercellular CO_2 partial pressure (C_i) equals approximately to CO_2 partial pressure at the site of carboxylation (C_c). However, intercellular CO_2 must diffuse within cell through chloroplast membrane to the site of carboxylation (Aalto and Juurola 2002, Gaastra 1959). Consequently, C_c would be lower than C_i (Harley *et al.* 1992a, Loreto *et al.* 1992, Niinemets *et al.* 2005, von Caemmerer 2000). Therefore, it is reasonable and important to couple the mesophyll conductance (g_m) limit into A/C_i curve analysis (Evans and Loreto 2000, Monti *et al.* 2006), especially when it comes to estimate the photosynthetic parameters. In a second assumption, parameters estimated from traditional A/C_i curve analysis or A/C_i model necessitate a prior C_i value at which the A/C_i curve switches between Rubisco- and electron transport-limited portions of the curve (C_i-t) and common values of C_i-t used for analysis range from 20 to 25 Pa (Harley *et al.* 1992a, Wullschleger 1993). However, some studies have pointed that C_i-t ranges greatly among plant species (Manter and Kerrigan 2004, Dubois *et al.* 2007). Consequently these parameters were estimated from A/C_i curve analysis with ignoring g_m limit and setting C_i-t a constant value (Bunce 2000, Leuning 2002, Medlyn *et al.* 2002, Wohlfahrt *et al.* 1999, Wullschleger 1993 *etc.*). Inevitably the assumptions of A/C_i curve analysis influenced the accuracy of parameter estimation.

A/C_c curve analysis is developed from A/C_i model through overwhelming the above two assumptions. Many studies have reported the effects of g_m or C_i-t on parameter estimation and developed new A/C_c methods (Ethier and Livingston 2004, Manter and Kerrigan 2004, Dubois *et al.* 2007, Miao *et al.* 2009 *etc.*). Ethier and Livingston (2004) and Manter and Kerrigan (2004) compared V_{cmax} from traditional A/C_i method and A/C_c method fitting with segmented A/C_c curve. Miao *et al.* (2009) point different A/C_c fitting methods can lead to the difference in parameter values and recommend to simultaneously fit parameters with the whole A/C_c curve. At present, relative few studies have quantified the relationship of parameters (V_{cmax}, J_{max}, and V_{cmax}/J_{max}) estimated from A/C_i and A/C_c curve analysis recommended by Miao *et al.* (2009). Therefore, based on the A/C_i curve data of *L. gmelinii* in northeast of China, the purpose of this study was to determine the effects of C_i-t and quantify the relationship between the parameters estimated from traditional A/C_i curve and A/C_c curve analysis directly fitting with the whole A/C_c curve, in order to provide accurate parameters for simulating the productivity and carbon gain of terrestrial ecosystems.

Materials and methods

Experimental site and materials: The experiment was executed at the boreal forest ecosystem research station in Hu Zhong nature preservation Region (122°42′ – 123°18′ E, 51°17′ – 51°56′ N), Heilongjiang province, China. The experiment region had an average elevation of 812 m, a mean monthly temperature of 15.95 ± 4.0°C from May to August, a mean annual precipitation of 511 mm and 85 growth days, featuring typical geographical and climate conditions.

L. gmelinii (Rupr.), 6 m high and 8 years old on average, was used in this experiment. The 30 selected trees lived with fertile soil and enough soil water since it often rained during the growing season.

Leaf gas exchange: Photosynthetic measurements of *L. gmelinii* leaves were taken on the newly formed mature sun leaves with a portable photosynthesis measurement system (*Li-6400*, *LI-COR*, *Inc.* Lincoln, NE, USA) between 08:30 and 12:00 h (local time), from May to August in 2007. 20 light-response curves were used to get the saturated light intensity. The light-response curves were measured at 1,450; 1,300; 1,150; 1,050; 900, 750, 600, 450, 350, 200, 100, 50, 0, 50, and 100 μmol m^{-2} s^{-1} photosynthetic photon flux densities (PPFD), with 380 μmol $mol^{-1}(CO_2)$ concentration, the leaf temperature of 25°C, and the relative humidity of 70%. In addition, 30 A/C_i curves were measured at 380, 250, 150, 50, 0, 50, 150, 300, 450, 600, 750, 900, and 1,050 μmol $mol^{-1}(CO_2)$ concentrations, with the PPFD of 1,100 μmol m^{-2} s^{-1}, the leaf temperature of 25°C, and the relative humidity of 70%.

Model description: FvCB model of C_3 plant (A/C_i curve analysis) can be described as (Farquhar *et al.* 1980, Harley and Sharkey 1991, Harley *et al.* 1992ab, Sharkey 1985):

$$A = V_\mathrm{c}(1 - \frac{\Gamma^*}{C_\mathrm{i}}) - R_\mathrm{D} \quad (1)$$

where A is the photosynthetic rate; V_c is the rate of carboxylation of Rubisco [μmol m^{-2} s^{-1}], equal to min{ A_c, A_j }; A_c and A_j are Rubisco- and electron transport-limited rates of carboxylation, respectively; Γ^* is the CO_2 compensation point in the absence of R_D.

Rubisco-limited photosynthesis is expressed as:

$$A_\mathrm{c} = \frac{V_\mathrm{cmax} C_\mathrm{i}}{C_\mathrm{i} + K_\mathrm{c}(1 + \frac{O}{K_\mathrm{o}})} \quad (2)$$

where O is the O_2 partial pressure in intercellular spaces [Pa]; K_c and K_o are the Michaelis-Menten constants of Rubisco activity for CO_2 and O_2, respectively.

The rate of photosynthesis limited by RuBP regeneration is expressed as:

$$A_\mathrm{j} = \frac{J C_\mathrm{i}}{4(C_\mathrm{i} + 2\Gamma^*)} \quad (3)$$

where J is the rate of electron transport [μmol electron m^{-2} s^{-1}] and can be described as (Farquhar *et al.* 1980, Harley *et al.* 1992b):

$$J = \frac{aI}{\sqrt{(1 + \frac{a^2 I^2}{J^2_\mathrm{max}})}} \quad (4)$$

where J_max is the maximum rate of electron transport; I is the incident irradiance; α is the quantum yield of electron transport [mol electrons mol^{-1} photon] (Harley *et al.* 1992a).

Here, the parameter estimation of A/C_c curve method took g_m limit into account and simultaneously fitted with the whole A/C_c curve. When photosynthetic rate is Rubisco-limited, the response of A to CO_2 concentration can be described by the following equation:

$$A = \frac{V_\mathrm{cmax}(C_\mathrm{c} - \Gamma^*)}{C_\mathrm{c} + K_\mathrm{c}\left(1 + \frac{O}{K_\mathrm{o}}\right)} - R_\mathrm{D} \quad (5)$$

where C_c is the CO_2 partial pressure at Rubisco.

When photosynthetic rate is limited by RuBP regeneration, A can be expressed as:

$$A = \frac{J(C_\mathrm{c} - \Gamma^*)}{4(C_\mathrm{c} + 2\Gamma^*)} - R_\mathrm{D} \quad (6)$$

where J is also calculated by Eq. 4.

C_c is related to C_i, A, and g_m. A/C_i curve data can be used to calculate g_m [μmol m^{-2} s^{-1} Pa^{-1}] through Eq. 5, 6 and the following equation:

$$C_\mathrm{c} = C_\mathrm{i} - \frac{A}{g_\mathrm{m}} \quad (7)$$

The temperature response of parameters was exponential. The equations can be expressed as (Harley *et al.* 1992b):

$$Parameter = e^{(c - \frac{\Delta H_\mathrm{a}}{R \bullet T})} \quad (8)$$

or

$$Parameter = \frac{e^{(c - \frac{\Delta H_\mathrm{a}}{R \bullet T})}}{1 + e^{(\Delta S \bullet T - \frac{\Delta H\mathrm{d}}{R \bullet T})}} \quad (9)$$

where c is a scaling constant, ΔH_a is enthalpy of activation [KJ mol^{-1}], ΔH_d is enthalpy of deactivation [KJ mol^{-1}], ΔS is entropy, and R is gas constant [8.314 J mol^{-1} K^{-1}] .

The constants used in A/C_i curve analysis, A/C_c curve analysis, and the temperature functions are in Table 1. Values of the parameters are taken from Bernacchi *et al.* (2001, 2002, 2003).

C_c-t or C_i-t can be obtained as follows by solving [$A_\mathrm{c} = A_\mathrm{j}$]:

$$C_\mathrm{c}\text{-t} = \frac{K_\mathrm{c} J_\mathrm{max}(K_\mathrm{o} + O) - 8 K_0 \Gamma^* V_\mathrm{cmax}}{K_\mathrm{o}(4 V_\mathrm{cmax} - J_\mathrm{max})} \quad (10)$$

Table 1. The constants used in A/C_i curve analysis, A/C_c curve analysis, and the temperature functions. ΔH_a – enthalpy of activation; ΔH_d – enthalpy of deactivation; ΔS – entropy.

Parameters	25°C	c	ΔH_a [kJ mol^{-1}]	ΔH_d [kJ mol^{-1}]	ΔS [kJ mol^{-1}K^{-1}]
Used for fitting					
K_c [Pa]	27.24	35.98	80.99		
K_o [kPa]	16.58	12.38	23.72		
Γ^*[Pa]	3.74	11.19	24.46		
Used for normalizing					
V_cmax	1	26.36	65.33		
J_max	1	17.71	43.9		
g_m	1	20.01	49.6	437.4	1.4

Model-fitting techniques and statistical analysis: Miao *et al.* (2009)'s *SA*S programs (grid search plus nonlinear, two-stage least square regression technique) (*SAS Institute Inc. 9.1*, Cary, NC, USA) were used to fit A/C_i curves and A/C_c curves. The optimum of V_{cmax}, J_{max}, R_D, and g_m were obtained based on the minimum of the root mean square error (RMSE) of each curve. In traditional A/C_i curve analysis, V_{cmax} and R_D were calculated through a portion of the A/C_i data where C_i is below a transition point (25 Pa), and then the whole A/C_i data were used to determine J_{max} by holding V_{cmax} and R_D values as constants (Harley *et al.* 1992a, Wullschleger 1993, Curtis *et al.* 1995). C_i-t was obtained by equation 10 with V_{cmax} and J_{max} that both were fitted simultaneously using all data of an A/C_i curve. In A/C_c curve method, V_{cmax}, g_m, and R_D were initially calculated using a variable transition point (C_c-t) where the regression mean square statistic was lowest, and then the whole A/C_c data were used to determine J_{max} by holding V_{cmax}, g_m, and R_D values as constants, finally refit all the four parameters simultaneously using the whole A/C_c data and inputting the initial V_{cmax}, J_{max}, g_m, and R_D values. It is important to point that C_c-t can be calculated through equation 10 by inputting V_{cmax}, J_{max}, g_m, and R_D which were estimated form A/C_c curve method, but these parameter estimations were independent of C_c-t or C_i-t.

Results

Effects of C_i-t on the parameters estimated from A/C_i curve analysis: Based on the equation 10, the C_i-t values for *L. gmelinii* were calculated and variable, ranging from 24 to 72 Pa, and its average value was 38 Pa. Furthermore, C_i-t value had important influence on parameter estimation of A/C_i curve analysis with segmented fitting method. As illustrated in Fig. 1, V_{cmax} estimated from A/C_i curve analysis was 11.3 μmol m^{-2} s^{-1} and J_{max} was 30.4 μmol m^{-2} s^{-1} when C_i-t was set 25 Pa (Fig. 1*A*); but V_{cmax} was 15.3 μmol m^{-2} s^{-1} and J_{max} was 44.7 μmol m^{-2} s^{-1} when C_i-t was set 72 Pa (Fig. 1*B*). Therefore, C_i-t would directly affect the parameter estimation from A/C_i curve analysis if C_i-t value was set too high or low.

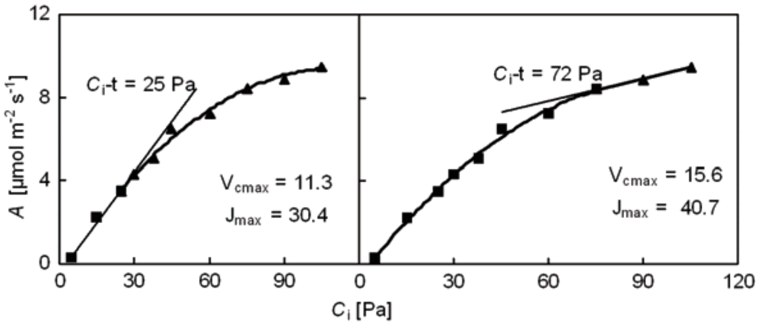

Fig. 1. Parameter estimation of A/C_i curve analysis when C_i transition point (C_i-t) is held as a constant (25 Pa) (Fig. 1*A*) and set maximum (72 Pa) (Fig. 1*B*), respectively. *Filled squares* represent the Rubisco-limited photosynthetic rate and *filled triangles* illustrate the electron transport-limited photosynthetic rate.

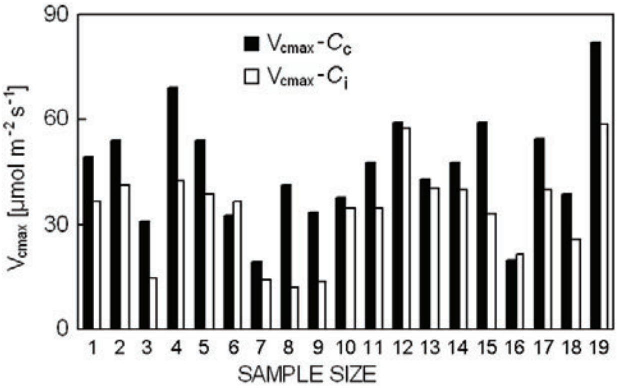

Fig. 2. Comparison between V_{cmax}-C_c and V_{cmax}-C_i. Black histograms represent V_{cmax} estimated from A/C_c curve analysis (V_{cmax}-C_c) and white histograms indicate V_{cmax} estimated from A/C_i curve analysis (V_{cmax}-C_i). *t*-test showed there were significant differences between V_{cmax}-C_c and V_{cmax}-C_i ($n = 19$, $p<0.01$), and V_{cmax}-C_i were lower than V_{cmax}-C_c on the whole.

Comparison of V_{cmax} based on A/C_i curve and A/C_c curve analysis: V_{cmax} estimated from A/C_i curve analysis (V_{cmax}-C_i) was based on the Rubisco-limited portion of A/C_i curve with a segmented fitting and ranged from 12 to 57.6 μmol m^{-2} s^{-1}. V_{cmax} from A/C_c curve analysis (V_{cmax}-C_c) ranged from 19.46 to 81.90 μmol m^{-2} s^{-1} and g_m ranged from 0.03 to 0.57 mol m^{-2} s^{-1}. From visual inspection in Fig. 2, most of the V_{cmax}-C_i values were lower than V_{cmax}-C_c values except one. The result of paired-sample *t*-test showed there was a significant difference between V_{cmax}-C_c and V_{cmax}-C_i ($p<0.01$). Comparing V_{cmax}-C_c with V_{cmax}-C_i, we found that mean V_{cmax}-C_i value was a significantly lower than V_{cmax}-C_c value on average. The mean V_{cmax}-C_c value of *L. gmelinii* was 45.95 μmol m^{-2} s^{-1} and V_{cmax}-C_i on average was 33.42 μmol m^{-2} s^{-1}. Compared with V_{cmax}-C_c, V_{cmax}-C_i was underestimated by 37.49% on average, ranging from –11.67% to 141.38%. Moreover, there was a significant linear relationship between them ($p<0.05$). As shown in

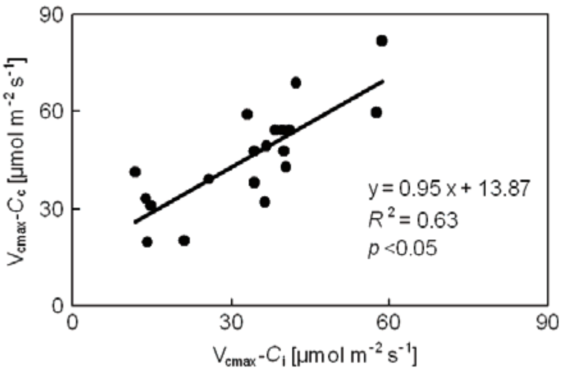

Fig. 3. Linear relationship between $\mathrm{V_{cmax}}$-C_c and $\mathrm{V_{cmax}}$-C_i ($p<0.05$). $\mathrm{V_{cmax}}$-C_c is $\mathrm{V_{cmax}}$ estimated from A/C_c curve analysis and $\mathrm{V_{cmax}}$-C_i is $\mathrm{V_{cmax}}$ estimated from A/C_i curve analysis.

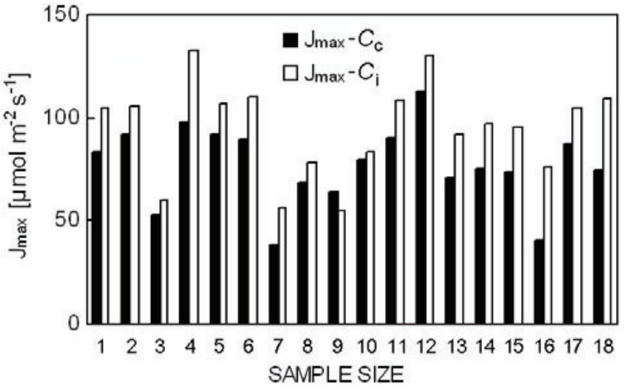

Fig. 4. Comparison between $\mathrm{J_{max}}$-C_c and $\mathrm{J_{max}}$-C_i. Black histograms represent $\mathrm{J_{max}}$ estimated from A/C_c curve analysis ($\mathrm{J_{max}}$-C_c) and white histograms indicate $\mathrm{J_{max}}$ estimated from A/C_i curve analysis ($\mathrm{J_{max}}$-C_i). *t*-test showed there were significant differences between $\mathrm{J_{max}}$-C_c and $\mathrm{J_{max}}$-C_i ($n = 18$, $p<0.01$), and $\mathrm{J_{max}}$-C_c were lower than $\mathrm{J_{max}}$-C_i on the whole.

Fig. 3, the slope of the regression equation between $\mathrm{V_{cmax}}$-C_i and $\mathrm{V_{cmax}}$-C_c was 0.95 which was close to 1 and its intercept was 13.87. Namely, $\mathrm{V_{cmax}}$-C_i value was about 13.87 μmol m^{-2} s^{-1} lower than $\mathrm{V_{cmax}}$-C_c.

Comparison of $\mathrm{J_{max}}$ based on A/C_i curve and A/C_c curve analysis: $\mathrm{J_{max}}$ estimated from A/C_i curve method ($\mathrm{J_{max}}$-C_i) was based on the whole A/C_i curve data by holding $\mathrm{V_{cmax}}$ and R_D values as constants and ranged from 54.9 to 133 μmol m^{-2} s^{-1}. $\mathrm{J_{max}}$ estimated from A/C_c curve analysis ($\mathrm{J_{max}}$-C_c) ranged from 38.15 to 112.46 μmol m^{-2} s^{-1} and g_m ranged from 0.03 to 0.57 mol m^{-2} s^{-1}. We found there was also a significant difference between $\mathrm{J_{max}}$-C_c and $\mathrm{J_{max}}$-C_i after paired-sample *t*-test ($p<0.01$). Comparing $\mathrm{J_{max}}$-C_c with $\mathrm{J_{max}}$-C_i, it showed that $\mathrm{J_{max}}$-C_c values were lower than $\mathrm{J_{max}}$-C_i values on the whole (Fig. 4). The average value of $\mathrm{J_{max}}$-C_c was 76.79 μmol m^{-2} s^{-1} and $\mathrm{J_{max}}$-C_i on average was 93.42 μmol m^{-2} s^{-1}. Compared with $\mathrm{J_{max}}$-C_c, $\mathrm{J_{max}}$-C_i was overestimated by 17.8% on average, ranging from –17.48% to 46.42%.

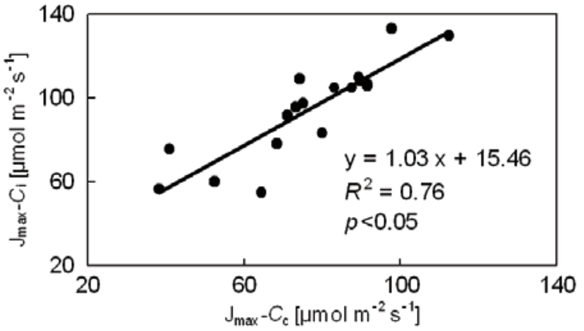

Fig. 5. Linear relationship between $\mathrm{J_{max}}$-C_c and $\mathrm{J_{max}}$-C_i ($p<0.05$). $\mathrm{J_{max}}$-C_c is $\mathrm{J_{max}}$ estimated from A/C_c curve analysis and $\mathrm{J_{max}}$-C_i is $\mathrm{J_{max}}$ estimated from A/C_i curve analysis.

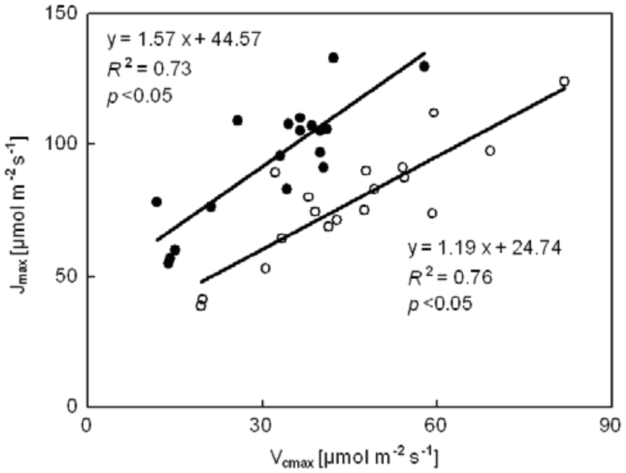

Fig. 6. Linear relationship between $\mathrm{J_{max}}$ and $\mathrm{V_{cmax}}$ ($p<0.05$). *Filled circles* represent linear relationship between $\mathrm{J_{max}}$ and $\mathrm{V_{cmax}}$ which were estimated from A/C_c curve analysis, and *open circles* represent linear relationship between $\mathrm{J_{max}}$ and $\mathrm{V_{cmax}}$ which were estimated from A/C_i curve analysis.

Furthermore, there was a significant linear relationship between them ($p<0.05$). As shown in Fig. 5, the slope of the regression equation between $\mathrm{J_{max}}$-C_i and $\mathrm{J_{max}}$-C_c was 1.03 which was close to 1 and its intercept was 15.46. It meant $\mathrm{J_{max}}$-C_i value was almost 15.46 μmol m^{-2} s^{-1} more than $\mathrm{J_{max}}$-C_c.

Comparison of $\mathrm{J_{max}/V_{cmax}}$ based on A/C_i curve and A/C_c curve analysis: As it has been reported (Wullschleger 1993), there was a consistent linear relationship between $\mathrm{V_{cmax}}$ and $\mathrm{J_{max}}$ (Fig. 6). Statistical result of *t*-test showed there was a significant difference between $\mathrm{J_{max}/V_{cmax}}$ based on A/C_i method and that based on A/C_c method ($p<0.01$). The slope of $\mathrm{J_{max}/V_{cmax}}$ based on A/C_i method was 1.57 and its intercept was 44.57 μmol m^{-2} s^{-1}. However, the slope of $\mathrm{J_{max}/V_{cmax}}$ based on A/C_c method was 1.19 and its intercept was 24.74 μmol m^{-2} s^{-1}. Obviously, the slope and intercept of $\mathrm{J_{max}/V_{cmax}}$ based on A/C_i method were overestimated and the slope was overestimated by 24.2%.

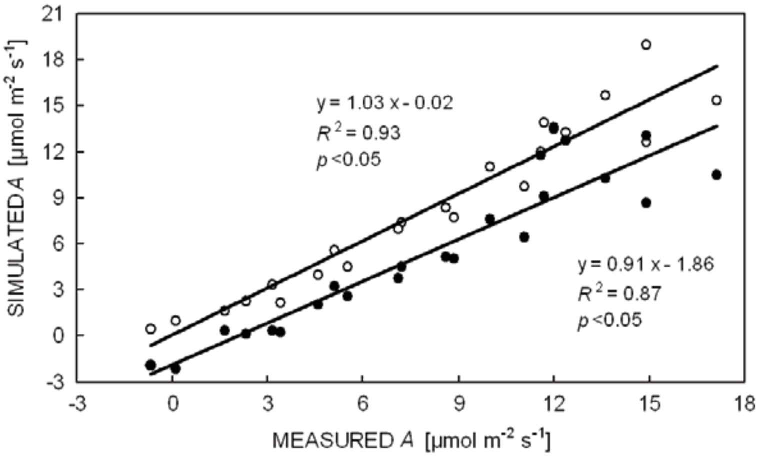

Fig. 7. Comparison between the simulated and the measured photosynthetic rates. *Filled circles* represent the relationship between the measured photosynthesis rates and the simulated values from A/C_i curve analysis (n = 23, $p<0.05$). *Open circles* indicate the relationship between the measured photosynthetic and the simulated values from A/C_i curve analysis (n = 23, $p<0.05$).

Comparison between the measured and simulated photosynthetic rates: Five A/C_i curves were repeatedly measured on the same tree. Three of the five curves were used to estimate parameters based on A/C_i and A/C_c methods, and then these parameters and the remaining curves were used to compare the predictive capabilities of A/C_i and A/C_c methods. Through comparing the measured photosynthetic rates of the two remaining A/C_i curves with the simulated values from A/C_i curve method and A/C_c curve method, our result showed that the slope of the regression equation between the simulated values from A/C_c curve method and the measured values was 1.03 which was close to 1, and the intercept was 0.02 which was close to zero ($p<0.05$; Fig. 7). Compared with the measured values, the simulated values from A/C_i curve method were a little lower, although A/C_i curve method could also simulate the photosynthetic rates well. Therefore, the simulated values from A/C_c curve method were closer to the measured values.

Discussion

In traditional A/C_i curve analysis, V_{cmax} and R_D were calculated through a portion of the A/C_i data where C_i is below a transition point (C_i-t), and then the whole A/C_i data were used to determine J_{max} by holding initial V_{cmax} and R_D values as constants (Harley *et al.* 1992a, Wullschleger 1993, Curtis *et al.* 1995). In general, C_i-t values used for analysis range from 20 to 25 Pa (Harley *et al.* 1992a, Wullschleger 1993). However, our analysis of *L. gmelinii*'s A/C_i curves showed that C_i-t value ranged from 24 to 72 Pa, averaging 38 Pa which was found lower than Manter and Kerrigan's mean value (71 Pa) after one-sample *t*-test ($p<0.01$). Furthermore, parameters estimated from A/C_i curve analysis when C_i-t was held as a constant (25 Pa) and set maximum (72 Pa) respectively were significantly different (Fig. 1). C_i-t value set too high or low could significantly influence the parameter estimation, which agrees with Manter and Kerrigan's conclusion (2004). From Eq. 10, C_i-t is a function of V_{cmax} and J_{max}, and plays an important role in parameter estimation. Hence it is unreasonable to use the segmented fitting or a constant C_i-t value in A/C_i or A/C_c curve analysis.

Based on A/C_i curve method and A/C_c curve method taking the effects of g_m limit and C_i-t on parameter estimation into account, our research quantified the relationship between parameters (V_{cmax}, J_{max} and V_{cmax}/J_{max}) respectively calculated from A/C_i curve method and A/C_c curve method. Our results showed that V_{cmax} from A/C_i curve analysis was underestimated by 37.49% on average than V_{cmax} from A/C_c curve analysis. However, the results from Manter and Kerrigan (2004) showed mean V_{cmax} calculated from A/C_i curve analysis was underestimated by 58.46%. The difference may be due to different g_m values and fitting methods of A/C_c curve. The A/C_c curve analysis used in their paper is a segmented fitting method, but here is direct and simultaneous fitting. Through the comparison of the A/C_c curve fitting methods, Miao *et al.* (2009) find different A/C_c fitting methods can lead to the significant difference in parameter values and recommend to simultaneously fit parameters with the whole A/C_c curve due to its fitting accuracy, simplicity of fitting procedures and sample size requirement. In addition, the materials used in Manter and Kerrigan's research were conifer seedlings potted in greenhouse, and our materials were high conifers in the field. Our results also found that J_{max} calculated from A/C_i curve analysis on average was overestimated by 17.8%. In A/C_i curve analysis, J_{max} were calculated under high CO_2 concentration, and the effect of g_m limit on J_{max} was marginal (Ethier and Livingston 2004). Therefore, the difference between J_{max} values calculated from A/C_i curve analysis and those calculated from A/C_c method may be due to the initial values of V_{cmax} and R_D used in J_{max} estimation processes. On the other hand, the traditional A/C_i curve analysis can lead to severe underestimation of Rubisco activity such that the entire A/C_i response curve can be apparently limited by Rubisco activity (Ethier and Livingston 2004). Consequently J_{max} was estimated most by high photosynthetic rates of A/C_i curve, and then overestimated by A/C_i curve analysis according to Eq. 3.

A/C_i curves data measured from May to August were used to estimate V_{cmax} and J_{max}, but this parameter values fluctuated greatly. There were two reasons at least. First, the different growth temperature can influence parameter values (such as J_{max}) (Bernacchi *et al.* 2003). We measured the A/C_i curves from May to August in 2007. The trees start to sprout in May when monthly mean temperature is near to 10°C, but August is the hottest month in the growth season of a year. Second, variable g_m can significantly influence parameter values. g_m can be influenced significantly by the total tree height, leaf N content, and leaf age (Niinemets *et al.* 2009). Here g_m values ranged greatly from 0.57 to 0.03 mol m^{-2} s^{-1}. Consequently the values of V_{cmax} and J_{max} fluctuated due to the variable g_m and environmental factors. Surprisingly, our analysis showed that there was a significant linear relationship between V_{cmax} calculated from A/C_i curve analysis and V_{cmax} calculated from A/C_c curve analysis, so was it J_{max} ($p<0.05$). It implied that these parameters from two different methods had the same responses to environmental factors. Obviously, g_m and C_i-t significantly influence the estimation of V_{cmax} and J_{max}, thus the correlations are closely related to g_m and C_i-t. Further studies are needed to find out if there is a co-adjustment between g_m and C_i-t to the above correlations.

The ratio of J_{max}/V_{cmax} is a key parameter that coordinates between Rubisco- and electron transport-limited photosynthetic processes, and has been comprehensively used in modelling seasonal dynamics in the photosynthesis of canopy. Through the analysis across 109 species, Wullschleger (1993) found a significant linear relationship occurred between V_{cmax} and J_{max}. It agreed with our results well. Our research also showed that the slope of J_{max}/V_{cmax} based on A/C_i curve analysis was 1.57, which equaled Centritto's result (2003) and close to 1.64 of Wullschleger's result (1993). However, the slope of J_{max}/V_{cmax} based on A/C_c curve analysis was 1.19. Consequently J_{max}/V_{cmax} based on A/C_i curve analysis was overestimated by 24.2% due to the underestimation of V_{cmax} and overestimation of J_{max}. It is important to note that many ecosystem models, such as DOLY (Woodward *et al.* 1995) and Biome-BGC (Thornton *et al.* 2002), set the slope of J_{max}/V_{cmax} to 1.64 according to Wullschleger's results (1993). Potential bias or errors of J_{max}/V_{cmax} may be exacerbated when the variable is scaled up from a single leaf to a canopy level, even to an ecosystem level. Therefore, those photosynthesis models of canopy and ecosystem models which are using the ratio of J_{max}/V_{cmax} should consider this point.

A/C_i method can not accurately estimate not only parameters but also photosynthetic rates. Due to inaccurate estimation of parameters and ignoring the effect of g_m, A/C_i method lead to comparatively poor simulations of photosynthetic rates in the field. It has been argued that even though A/C_i curve analysis is logically biased since it ignores g_m, it remains useful for simulation of photosynthesis. The present modelling study clearly showed that this pragmatic view was flawed insofar as A/C_i method is not as good as A/C_c method in simulating photosynthesis.

Since FvCB model has been incorporated into carbon exchange models, accurate parameter estimation is getting more and more meaningful. However, many present parameter estimation and model parameterizations are unreasonable because of ignoring the effects of g_m limit and C_i-t on parameter estimation. Though the empirical parameterizations based on FvCB model remain useful to predict photosynthesis, they may mislead us when it comes to evaluate the variability of parameters among C_3 plant species or to interpret the fundamental physiological processes underlying the measured photosynthetic responses of plants to various environmental conditions or through time (Ethier and Livingston 2004). Considering the importance of parameters in global carbon budget modelling, it is necessary and urgent to improve the accuracy of the parameter estimation.

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