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Quantifying the measurement errors in a portable open gas-exchange system and their effects on the parameterization of Farquhar *et al.* model for C_3 leaves

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Abstract

A portable open gas-exchange system (*Li-6400, Li-Cor, Inc.*, Lincoln, NE, USA) has been widely used for the measurement of net gas exchanges and calibration/parameterization of leaf models. Measurement errors due to diffusive leakage rates of water vapor (L_W) and CO_2 (L_C) between inside and outside of the leaf chamber, and the inward dark transpiration rate (D_W) and dark respiration rate (D_C) released from the leaf under the gasket, can be significant. Rigorous model-based approaches were developed for estimating leakage coefficients of water vapor (K_W) and CO_2 (K_C) and correcting for the combination of these errors. Models were based on mass balance equations and the Dusty Gas Model for a ternary gas mixture of water vapor, CO_2, and dry air. Experiments were conducted using two *Li-6400* systems with potato and soybean leaves. Results indicated that models were reliable for estimating K_W and K_C, and the values varied with instrument, chamber size, gasket condition, and leaf structure. A thermally killed leaf should be used for this determination. Measurement error effects on parameterization of the Farquhar *et al.* (1980) model as determined by P_N/C_i curves were substantial and each parameter had its own sensitivity to measurement errors. Results also indicated that all four error sources should be accounted for when correcting measurements.

Additional key words: open gas-exchange system; measurement errors; parameterization; leaf model; photosynthesis; transpiration.

Introduction

Portable open gas-exchange analysis systems with leaf chambers (*e.g., LI-6400, Li-Cor, Inc.*, Lincoln, NE, USA) have been widely used for the measurement of net exchange rate of H_2O (E) and CO_2 (P_N), the response of P_N to environment and intercellular CO_2 mole fraction (C_i), and calibration and parameterization of leaf models of E, P_N, and stomatal conductance (g_s). The open gas system is a device in which there is a net air flow through the system. Typical open system consists of four major parts: (*1*) an air supply unit, where air humidity, CO_2 concentration, temperature, *etc.* can be established by some means prior to entering the cuvette, (*2*) a flow meter

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Abbreviations: $\overline{A_{as}}$ – mean mole fraction of dry air; C – CO_2 concentration; C_a – ambient air CO_2 mole fraction; C_{AM} – total air mixture concentration; C_i – intercellular CO_2 mole fraction; C_r – reference CO_2 mole fraction; C_s – sample CO_2 mole fraction; $\overline{C_{as}}$ – mean CO_2 mole fraction; DGM – Dusty Gas Model; D_{wa} – binary diffusivity for water and air; D_{cw} – binary diffusivity for water and CO_2; D_{ac} – binary diffusivity for CO_2 and air; D_W^c – Knudsen diffusivity for CO_2; D_W^k – Knudsen diffusivity for water; D_C – rate of dark respiration escape from the leaf under gasket; D_W – rate of dark transpiration escape from the leaf under gasket; E – water vapor exchange rate; E_A – apparent water vapor exchange rate; E_D – dark water vapor exchange rate; E_{DA} – apparent dark water vapor exchange rate; F_i – incoming air mole flow rate; F_o – outgoing air mole flow rate; g_b – boundary layer conductance for H_2O; g_s – stomatal conductance for water vapor; IRGA – infrared gas analyzer; J_{max} – maximum rate of electron transport; K_C – diffusion leakage coefficient of CO_2; K_W – diffusion leakage coefficient of H_2O; L_A – diffusion leakage rate of dry air; L_C – diffusion leakage rate of CO_2; L_W – diffusion leakage rate of water; n_{CO2} – number of moles of CO_2; n_{da} – number of moles of dry air; N_{H2O} – number of moles of water vapor; P_{am} – pressure of the air mixture; P_N – net CO_2 exchange rate; P_{NA} – apparent net CO_2 exchange rate; r – ratio of inward gas exchange to total gas exchange of the leaf under gasket; R – universal gas constant; R_D – dark respiration rate; R_{DA} – apparent dark respiration rate; R_L – respiration in the light rate; S – window area of leaf chamber; T – temperature of the leaf chamber; TPU – rate of use of triose phosphates; V – volume of the leaf chamber; V_{cmax} – maximum rate of Rubisco activity-limited carboxylation; W – water vapor concentration; W_a – ambient water vapor mole fraction; W_r – reference water vapor mole fraction; W_s – sample water vapor mole fraction; $\overline{W_{as}}$ – mean mole fraction of water; Δl – diffusion effective length.

for precisely measured incoming air flow rate (F_i), (*3*) a leaf chamber, usually a clamp-on type with four gaskets: two adhered to the upper and lower part of the leaf chamber clamped onto a living leaf, and two air seal gaskets located behind the chamber gaskets, and (*4*) the infrared gas analyzers (IRGA) for measuring water vapor and CO_2 mole fraction in the incoming air flow (W_r and C_r) and outgoing air flow (W_s and C_s). Air from the air supply is split into two air flows: one to the reference cell of IRGA, another one, measured by the air flow meter through the leaf chamber, to the analysis cell of IRGA. The measurements are based on the differences in H_2O and CO_2 between incoming air flow and outgoing flow.

In practice, when the measurements are performed, two groups of measurement errors will inevitably occur to alter the real E and P_N. One group includes the diffusive leakage rates of H_2O (L_W) and CO_2 (L_C) through the air pathway from the air flow meter to the sample cell of IRGA, *i.e.* through chamber foam gaskets (Long and Hällgren 1993) and the air seal gaskets, air pores between gaskets (or between leaf and gasket if a leaf enclosed) (Flexas *et al*. 2007, Rodeghiero *et al*. 2007), O-rings and other materials (that are not glass or metal) (*Li-Cor* 2008). Another group includes the effects of inward dark transpiration (E_D) and dark respiration (R_D) released by the leaf portion under the gasket (Pons and Welschen 2002). The leakages are especially significant when large H_2O and CO_2 gradients are established between inside and outside the leaf chamber (*e.g.,* the response of P_N at the lower and higher values of C_i during measurement of P_N/C_i curves), or when E and P_N are small (*e.g*., the plant is under environmental stress). Unfortunately, attempts to eliminate or minimize leakages are difficult and have largely failed (Flexas *et al.* 2007). As a result, these measurement errors are often ignored (Pons and Welschen 2002) and it has been incorrectly argued that leakages are not a significant problem with open systems because the chamber is slightly pressured (McDermitt *et al*. 2001, *Li-Cor* 2008). Long and Hällgren (1993), Long and Bernacchi (2003), McDermitt *et al*. (2001), Bernacchi *et al*. (2001), Flexas *et al*. (2007), Rodeghiero *et al.* (2007), and *Li-Cor* (2008) have attempted to estimate L_C and its effects on measurements in the open gas-exchange system. These results demonstrated the significance of L_C and its substantial effect on P_N/C_i curves, and/or parameterization of Farquhar *et al*. (1980) model.

However, each of these methods were based on theoretically incomplete approaches and leakage correction results were inconsistent. *Li-Cor* (2008) and McDermitt *et al.* (2001) used an empty chamber to estimate CO_2 leakage coefficient (K_C) and corrected apparent photosynthesis rate (P_{NA}) by directly subtracting L_C from P_{NA}. Long and Bernacchi (2003) argued the leakage was increased if a leaf was enclosed within a leaf chamber, and suggested that using a dried leaf to estimate the leakage coefficient at each CO_2 concentration should be used in correction of P_N/C_i response. Flexas *et al.* (2007) stated that the structure of a thermally killed leaf was closer to a living leaf than the dried leaf and observed a substantially lower K_C with a thermally killed leaf as compared to an empty chamber. They corrected P_{NA} by simple subtraction of the relationship between C_s and P_{NA}. Flexas *et al.* (2007) also concluded that L_C resulted in an increase up to 40% for day respiration (R_L) and a 10% overestimation of the maximum capacity for carboxylation (V_{cmax}); however, this error generally did not significantly affect the maximum capacity for electron transport rate (J_{max}). However, none of the studies mentioned above incorporated the effects of L_W, E_D, and R_D on P_N and g_s.

Based on the combination of Fick's law and mass balances, Rodeghiero *et al.* (2007) derived different equations for calculation of K_C and the H_2O leakage coefficient (K_W) for an empty chamber and a chamber with a dried leaf, and correction expressions for both apparent transpiration (E_A) and P_{NA}. Their study was the only study to determine K_W and the effect of L_W on L_C. Their observations suggested that K_C increased, and a K_W determination was more variable, if a dried leaf was used, and thus empty leaf chamber estimates of K_C and K_W could not be used to correct E_A and P_{NA}. They also stated that the effect of K_W on K_C increased with increasing C_s up to 60% at C_s of 2,000 μmol mol^{-1}. Their sensitivity analysis showed that L_C would overestimate parameters of Farquhar *et al.* (1980) model in the order of $V_{cmax} < J_{max} < R_L$. The L_W further enhanced the overestimation of the model parameters. They observed the effects L_W on P_N/C_i curves that were at least as much as CO_2 diffusion. However, their study did not consider the effects of inward E_D and R_D produced by the leaf under the gasket.

Calculations of E_A and P_{NA} assume that these gas-exchange processes only occur in the portion of leaf filled in the leaf chamber (*Li-Cor* 2008). However, E_D and R_D from the portion of the leaf under the gasket continue after the leaf is clamped and H_2O and CO_2 may continually enter the chamber to alter W_s and C_s, and outgoing air flow rate (F_o) leading to substantial measurement errors, especially when E and P_N are low. The pathway for these gasses is mainly along the interface between the gasket and leaf surface but to a lesser extent includes leakage through the leaf mesophyll and gasket itself (Jahnke and Krewitt 2002, Pons and Welschen 2002, Flexas *et al.* 2007). Pons and Welschen (2002) observed an overestimation of R_D by 55%, exactly the ratio of the inward gasket area to the area of leaf chamber (their estimation of this area ratio was inaccurately computed as 59%), as a result of the rate of dark respiration contributed from the portion of the leaf clamped under the gaskets to the interior of the leaf chamber (D_C). The effect of inward E_D (D_W) has not been studied so far.

Neither of the investigations mentioned above were theoretically correct. The problems were not only a result of their incomplete consideration for two groups of errors, but also due to their calculations of K_C and K_W

from the chamber clamped on a dead leaf (dried or thermally killed leaf), and the correction methods for these measurement errors. They did not take the air leakage rate (L_A) into account in their mass balance equation and their leakage equations were derived based on Fick's law. However, Fick's law is valid strictly for isothermal, isobaric and equimolar countercurrent diffusion of a binary gas mixture. Unlike Fick's law, which is empirical, the Dusty Gas Model (DGM) is based on the Chapman Enskog kinetic theory of gases. The DGM is a fundamental approach to gas diffusion in the porous media (Mason and Malinauskas 1983). The DGM includes the Stefan-Maxwell formulation and takes into account Knudsen diffusion. It treats the porous medium as a component of the gas mixture, consisting of giant molecules, like dust in a gas. The DGM can be adapted to more accurately describe the leakages of H_2O and CO_2 through the leaf chamber.

In order to obtain the accurate measurements and modeling of gas exchanges of H_2O and CO_2, it is necessary to estimate K_W and K_C, and D_W and D_C, and their effects for any particular leaf under any particular experimental conditions. Quantifying and modeling such effects are needed to revise the existing correction approaches for measurement errors. The objectives of this study are (*1*) to outline more rigorous model-based approaches for estimating leakage coefficients using the DGM and mass balance equations, (*2*) to develop methods for correcting H_2O and CO_2 exchange rates, (*3*) to estimate the effects of these measurement errors on the parameterization of the C_3 leaf model of Farquhar *et al.* (1980).

Materials and methods

Model description: Since there are the H_2O and CO_2 mole gradients between inside and outside the leaf chamber, and inward E_D and R_D from the portion of leaf under the gasket, H_2O and CO_2 must enter or escape the leaf chamber. In the following sections we provide working equations to determine these leakages and to correct the measurement. The detailed derivation of all equations is given in the Appendix and a list of symbols is provided in Table 1.

(1) Diffusive leakage coefficients of an empty chamber

At steady-state condition, the working equations for K_W and K_C of an empty chamber are:

$$K_W = \frac{F_i\,(W_s - W_r)}{(W_a - W_s)} \tag{1}$$

and

$$K_C = \frac{F_i\,(C_r - C_s)}{(C_s - C_a)} \tag{2}$$

(2) Leakage coefficients of the chamber with a dead leaf

Because a very dry leaf might be a sink of water vapor and a wet leaf could be a source of water vapor, the leakage coefficient of water should be determined when leaf water is balanced with the ambient air. Under this condition, the leakage coefficient of water vapor can be given by Eq. 1. The working equation for estimating leakage of CO_2 is:

$$K_C = \frac{100\ P_{NA}\,S - \frac{K_W\,(W_s - W_a)\,C_s}{1000 - W_s}}{C_s - C_a} \tag{3}$$

(3) Correction for the measurement errors

The working equations for correction of the measurement errors for water vapor and CO_2 leakages are:

$$E = E_A + \frac{10\,K_W\,(W_s - W_a)}{S\,(1000 - W_s)} - r\,E_D \tag{4}$$

and

$$P_N = P_{NA} - \frac{K_W\,(W_s - W_a)\,C_s}{100\,S\,(1000 - W_s)} + \frac{K_C\,(C_a - C_s)}{100\,S} - r\,R_D \tag{5}$$

(4) Calculation of g_s and C_i

Because of the correction of the measurement errors, g_s and C_i have to be recalculated according to corrected E and P_N. In this paper, we treat only two particular cases: the hypostomatous leaf where only one side of the leaf has stomata, and the symmetrical amphistomatous leaf where physiological and environmental conditions are identical in both leaf sides, *i.e.* W_i and W_a, C_i and C_a, stomatal conductance for water vapor (g_s) and boundary layer conductance (g_b) are the same. Following von Caemmerer and Farquhar (1981), for hypostomatous leaf, g_s can be estimated by:

$$g_s = \frac{\left(1 - \frac{W_i + W_s}{2000}\right) g_b\,E}{(W_i - W_s)\,g_b - \left(1 - \frac{W_i + W_s}{2000}\right) E} \tag{6a}$$

C_i is given by

$$C_i = \frac{\left(\frac{g_s\,g_b}{1.6\,g_b + 1.37\,g_s} - \frac{E}{2000}\right) C_s - P_{N\,n}}{\frac{g_s\,g_b}{1.6\,g_b + 1.37\,g_s} + \frac{E}{2000}} \tag{7a}$$

where 1.6 and 1.37 are the ratio of the stomatal conductance to H_2O and to CO_2 in air and in the boundary layer, respectively. For the symmetrical amphistomatous leaf, g_s and C_i are given respectively by:

$$g_s = \frac{\left(1 - \frac{W_i + W_s}{2000}\right) g_b\,E}{2\,(W_i - W_s)\,g_b - \left(1 - \frac{W_i + W_s}{2000}\right) E} \tag{6b}$$

and

$$C_i = \frac{\left(\frac{g_s\,(2\,g_b)}{1.6\,(2\,g_b) + 1.37\,g_s} - \frac{E}{2000}\right) C_s - P_N}{\frac{g_s\,(2\,g_b)}{1.6\,(2\,g_b) + 1.37\,g_s} + \frac{E}{2000}} \tag{7b}$$

Table 1. List of model variables and their units.

Variable	Definition	Unit
$\overline{A_{as}}$	Mean mole fraction of dry air	[mmol(air) mol^{-1}]
P_N	Net CO_2 exchange rate	[μmol(CO_2) m^{-2} s^{-1}]
P_{NA}	Apparent net CO_2 exchange rate	[μmol(CO_2) m^{-2} s^{-1}]
C	CO_2 concentration	[mol(CO_2) m^{-3}]
C_a	Ambient air CO_2 mole fraction	[μmol(CO_2) mol^{-1} (air)]
C_{AM}	Total air mixture concentration	[mol m^{-3}]
C_i	Intercellular CO_2 mole fraction	[μmol(CO_2) mol^{-1}(air)]
C_r	Reference CO_2 mole fraction	[μmol(CO_2) mol^{-1}(air)]
C_s	Sample CO_2 mole fraction	[μmol(CO_2) mol^{-1}(air)]
$\overline{C_{as}}$	Mean CO_2 mole fraction	[μmol(CO_2) mol^{-1}(air)]
D_{wa}	Binary diffusivity for water and air	[m^2 s^{-1}]
D_{cw}	Binary diffusivity for water and CO_2	[m^2 s^{-1}]
D_{ac}	Binary diffusivity for CO_2 and air	[m^2 s^{-1}]
D_W^c	Knudsen diffusivity for CO_2	[m^2 s^{-1}]
D_W^k	Knudsen diffusivity for water	[m^2 s^{-1}]
D_C	Rate of dark respiration escape from the leaf under gasket	[μmol(CO_2) m^{-2} s^{-1}]
D_W	Rate of dark transpiration escape from the leaf under gasket	[mmol (CO_2) m^{-2} s^{-1}]
E	Water vapor exchange rate	[mmol(H_2O) m^{-2} s^{-1}]
E_A	Apparent water vapor exchange rate	[mmol(H_2O) m^{-2} s^{-1}]
E_D	Dark water vapor exchange rate	[mmol(H_2O) m^{-2} s^{-1}]
E_{DA}	Apparent dark water vapor exchange rate	[mmol(H_2O) m^{-2} s^{-1}]
F_i	Incoming air mole flow rate	[μmol(air) s^{-1}]
F_o	Outgoing air mole flow rate	[μmol(air) s^{-1}]
g_b	Boundary layer conductance for H_2O	[mol(H_2O) m^{-2} s^{-1}]
g_s	Stomatal conductance for water vapor	[mol(H_2O) m^{-2} s^{-1}]
J_{max}	Maximum rate of electron transport	[μmol(e)$^-$ m^{-2} s^{-1}]
K_C	Diffusion leakage coefficient of CO_2	[μmol(CO_2) s^{-1}]
K_W	Diffusion leakage coefficient of H_2O	[μmol(H_2O) s^{-1}]
L_A	Diffusion leakage rate of dry air	[mmol(air) s^{-1}]
L_C	Diffusion leakage rate of CO_2	[μmol(CO_2) s^{-1}]
L_W	Diffusion leakage rate of water	[mmol(H_2O) s^{-1}]
n_{CO2}	Number of moles of CO_2	
n_{da}	Number of moles of dry air	
N_{H2O}	Number of moles of water vapor	
P_{am}	Pressure of the air mixture	[Pa]
r	Ratio of inward gas exchange to total gas exchange of the leaf under gasket	
R	Universal gas constant	8.314 J mol^{-1} K^{-1}
R_D	Dark respiration rate	[μmol(CO_2) m^{-2} s^{-1}]
R_{DA}	Apparent dark respiration rate	[μmol(CO_2) m^{-2} s^{-1}]
R_L	Respiration in the light rate	[μmol(CO_2) m^{-2} s^{-1}]
S	Window area of leaf chamber	[cm^{-2}]
T	Temperature of the leaf chamber	[K]
TPU	Rate of use of triose phosphates	[μmol(CO_2) m^{-2}s^{-1}]
V	Volume of the leaf chamber	[m^3]
V_{cmax}	Maximum rate of Rubisco activity-limited carboxylation	[μmol(CO_2) m^{-2}s^{-1}]
W	Water vapor concentration	[mol(H_2O) m^{-3}(air)]
W_a	Ambinet water vapor mole fraction	[mmol(H_2O) mol^{-1}(air)]
W_r	Reference water vapor mole fraction	[mmol(H_2O) mol^{-1}(air)]
W_s	Sample water vapor mole fraction	[mmol(H_2O) mol^{-1}(air)]
$\overline{W_{as}}$	Mean mole fraction of water	[mmol(H_2O) mol^{-1}(air)]
Δl	Diffusion effective length	[m]

Eq. 6b is very similar to Eq. 6a, except that the factor 2 arises in the first term of the denominator as a result of use of g_s defined on a single surface area basis for symmetrical amphistomatous leaf. The magnitude g_s from Eq. 6b is half of the corresponding value estimated by equation provided by *Li-Cor* (2008), where g_s was defined as combined conductance for stomatal conductance in both sides of the leaf. (Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the USDA and does not imply the exclusion of other available products.)

(5) Calculation of parameters of photosynthesis biochemical model

After correction of P_N/C_i curves, the parameters of the Farquhar *et al.* (1980) model for C_3 plant, the maximum velocity of Rubisco for carboxylation (V_{cmax}), the maximum rate of electron transport (J_{max}); the rate of use of triose phosphates (TPU) and the respiratory CO_2 release other than by photorespiration (primarily mitochondrial respiration) (R_L), were estimated by fitting method as suggested by Sharkey *et al.* (2007).

Plant materials: Two C_3 plants, potato (*Solanum tuberosum* L. cv. Kennebec) with hypostomatous leaves (Pachepsky and Acock 1998), and soybean (*Glycine max* Merr. cv. Kent), with amphistomatous leaves (Bunce 2006), were used for the experiments. Potato plants were grown in 16-L pots with a 3:1 mixture of sand:vermiculite and soybeans in 2-L pots with vermiculite. Pots were fertigated until free drainage from the bottom of the pot daily using half-strength Woody's solution (Robinson 1984). Soybeans were initially grown in controlled environment chambers at a 1,000 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) for 12 h per day (43.2 mol PAR m^{-2} d^{-1}), with constant air temperature of 25°C. Following reproductive stage R3, soybeans were moved to an outdoor pad where conditions averaged 44.2 ± 17 mol PAR m^{-2} d^{-1}, 18.7 ± 4.8°C, and relative humidity ranged between 50 and 100% for the remainder of the study. Potatoes were grown on the same outdoor pad where conditions averaged 44.1 ± 15.8 mol PAR m^{-2} d^{-1}, 16.2 ± 5.3°C, and relative humidity ranged between 45 and 100%. These were typical conditions for the months of April, May, and June in Beltsville, MD, USA. Soybeans were at the R3 stage during measurements and potatoes were approximately 21 days post tuber initiation. Mature, fully green leaves within seven days of achieving full expansion were used for leaf measurements for both plants (second and third trifoliate leaves for soybeans and fourth or fifth leaf from top of the canopy for potato).

For homobaric leaves with significant lateral gas exchange (Jahnke 2001, Jahnke and Krewitt 2002, Pieruschka *et al.* 2005, 2006), determination of the ratio of inward gas exchange to the total gas exchange of the leaf under gasket is difficult. However, Flexas *et al.* (2007) and Morison and Lawson (2007) argued that the lateral diffusion was much smaller than the leakage between the leaf and gasket surface. In this study, for simplification, we assumed both potato and soybean leaves were heterobaric leaves.

Determining H_2O and CO_2 leakage coefficients: The experiments were conducted in the laboratory using two *Li-6400* systems equipped with either a 2 cm^2 fluorometer head model *6400-40* or a 6 cm^2 head *6400-02B* (*Li-Cor, Inc.,* Lincoln, NE, USA). One was used for gas-exchange measurements and another was used for monitoring the ambient air mole fractions of water vapor and CO_2 in the immediate vicinity of the leaf chamber. Instruments were marked as instrument-A and -B. Before measurements, the two instruments were calibrated for CO_2 using standard gases with CO_2 mole fraction of 0, 361, 727, and 1,004 μmol mol^{-1} according to the procedure suggested by the manufacturer (*Li-Cor* 2008). For the water vapor calibration, the H_2O zero of instrument IRGAs were checked daily using chemical methods as suggested by the manufacturer (*Li-Cor* 2008). Both instruments were initially compared against one another with respect to the same ambient air and measured water vapor readings were less than 0.3 mmol mol^{-1}. The lower gasket of both leaf chambers was made of black neoprene foam, the upper white gasket was made of white polyethylene foam as provided by the manufacturer.

The leakage coefficients in instrument-B with a worn gasket (which had been previously used for at least 40 h) were obtained by three series of measurements. These included (*1*) an empty chamber, (*2*) chamber clamped on a dried leaf, or (*3*) chamber clamped on a thermally killed leaf. The block temperature was set to 25–30°C similar to the ambient air temperature. Note that the leakage coefficients vary with the 1.5 to 1.8 power of temperature (Cussler 2007), but at this temperature range, the effects are very small, about 3%. At each measurement, we waited until steady-state conditions were observed, then the IRGAs were matched before recording the data. Each measurement was replicated at least three times. To get large water vapor and CO_2 gradients for estimating K_C and K_W, CO_2-response curves were performed under different water vapor gradient conditions. These curves were obtained using two different leaf chambers: 2 and 6 cm^2. During these measurements, external ambient CO_2 concentration varied between 385 and 560 μmol mol^{-1} and ambient water vapor varied between 10 and 25 mmol mol^{-1}.

To compare the leakage coefficient difference between the two instruments, additional experiments were conducted in instrument-A with or without a dead leaf filled in the 6 cm^2 leaf chamber. The differences of leakage coefficients for different gasket wear conditions were examined by an additional experiment conducted using new gaskets with a thermally killed leaf inside the 6 cm^2 chamber. To examine the possible effect of leaf thickness on K_C as noted by Flexas *et al.* (2007), we rehydrated a dried leaf with distilled water for 2 h to make the leaf thicker and the structure closer to a living leaf. This effect was measured by the measurements with the dried leaf *vs.* the rehydrated leaf.

Determining the gas exchanges of potato and soybean leaves: The results reported in the following sections were estimated using instrument-B with worn gaskets unless otherwise noted. To get the desired water vapor gradient, when water vapor mole fraction in ambient air was high, the valve of the desiccant tube was adjusted to decrease the water vapor mole fraction inside the chamber for negative gradients of –25 and –15 mmol mol^{-1}; and when ambient water vapor mole fraction was low, a few drops of distilled water were added to the soda lime tube to increase water vapor mole fraction inside the chamber in order to get the desired gradient of 15 mmol mol^{-1}. The leaf gas exchange was determined using two *Li-6400* instruments with a similar procedure as described already for estimating K_W and K_C. The measurements were made on the youngest fully expanded leaves. The E_D and R_D rates of potato and soybean were measured using the 6 cm^2 chamber. After achieving the steady state, the IRGA's were matched and data recorded.

To quantify the effects of measurement errors induced by L_W and L_C, and D_W and D_C on parameterization of the Farquhar *et al.* (1980) for C_3 plants, P_N/C_i curves were determined in potato and soybean fully expand mature leaves under different W_s. The leaf temperature was set close to ambient air temperature. The measurements were performed using 2 and 6 cm^2 chambers. To obtain large water vapor gradient between inside and outside leaf chamber, the high reference water vapor mole fraction was achieved by adding a few drops of distilled water to the soda lime and the incoming air would be humidified through the bypass valve of the desiccant tube. The high ambient air humidity was achieved by conducting the measurements inside an outdoor sunlit growth chamber (Fleisher *et al.* 2008). The water vapor gradient ranged from –15 to +20 mmol mol^{-1}. The measurements were replicated at least 3 times at different water vapor gradients using two different size chambers.

Results

Leakage coefficients of the empty chamber: The slopes of linear regression relationships $\mathrm{F_i}\,(\mathrm{W_s} - \mathrm{W_r})$ *vs.* $(\mathrm{W_a} - \mathrm{W_s})$ (Eq. 1), and $\mathrm{F_i}\,(C_\mathrm{r} - C_\mathrm{s})$ *vs.* $(C_\mathrm{s} - C_\mathrm{a})$ (Eq. 2) were estimates of K_W and K_C, respectively, as shown in Fig. 1 in instrument-B for the worn gasket. Overall, K_W was 4.34 μmol s^{-1} in the 2 cm^2 chamber and 3.60 μmol s^{-1} in the 6 cm^2 chamber, K_C was 0.42 μmol s^{-1} in the 2 cm^2 chamber and 0.46 μmol s^{-1} in the 6 cm^2 chamber. Results for instrument-A with a 6 cm^2 chamber and worn gasket included a K_W of 6.75 μmol s^{-1} and K_C of 0.94 μmol s^{-1} (data not shown).

Effect of presence of a leaf: Fig. 2 showed two representative examples of changes in calculated K_W (Eq. 3) *vs.* time for a dried and a thermally killed potato leaf by instrument-A. When the interior air of the leaf chamber is more humid than the exterior air, a clamped dried potato leaf will absorb H_2O from its environment, resulting

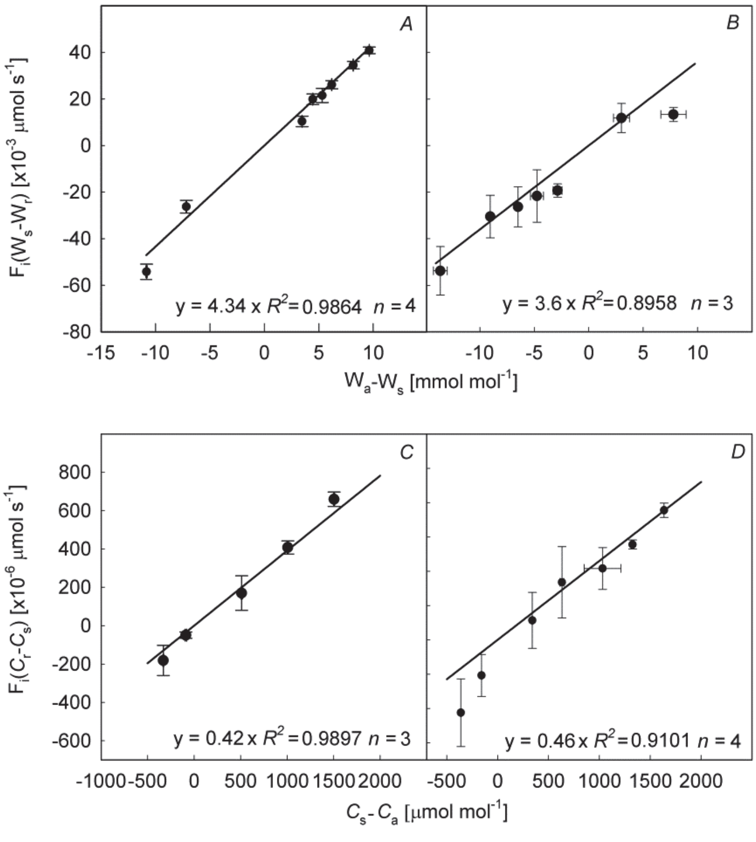

Fig. 1. The response of leakages of water vapor (*A, B*) and CO_2 (*C, D*) to the mole fraction gradients between inside and outside an empty chamber in instrument-B fitted with a worn gasket. Graphs *A* and *C* showed results for the 2 cm^2 chamber, and *B* and *D* the 6 cm^2 chamber. Values are the mean ± SD (*n* = 3 or 4).

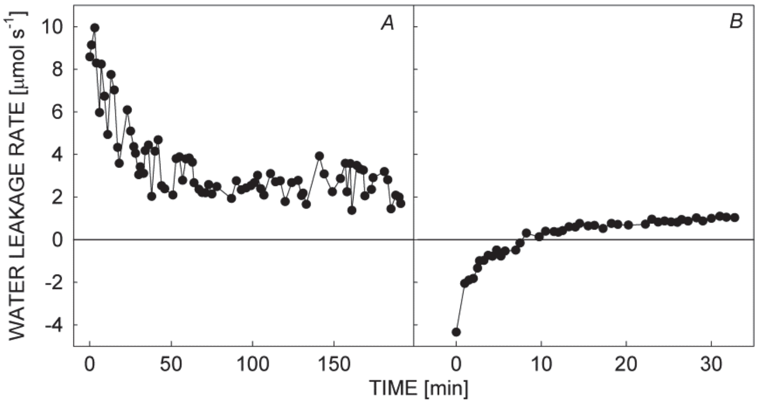

Fig. 2. Changes in calculated water vapor leakage coefficient with time for instrument-A. The chamber size was 6 cm^2 with worn gaskets. A dried potato leaf was used inside a more humid leaf chamber in graph *A*, and a thermally killed one inside a drier leaf chamber in graph *B*. Data are shown for a single leaf as an illustration.

Table 2. Leakage coefficients of water (K_W) and CO_2 (K_C) for a dead leaf (thermally killed leaf or dried leaf) determined by instrument-A or -B with worn gaskets. The K_W was determined as the slope of regression when the leaf water was balanced with ambient air. The values of K_W were means ± SD. The values of K_C were the slope of linear regression relationship $100\,P_{\mathrm{NA}}\,\mathrm{S}-\frac{\mathrm{K_W}\,(\mathrm{W_s}-\mathrm{W_a})\,C_\mathrm{s}}{1000-\mathrm{W_s}}$ *vs.* $(C_\mathrm{s}-C_\mathrm{a})$ (Eq. 21a) followed by correlation coefficients (*n* = 3). [a] – thermally killed leaf with worn gaskets; [b] – dried leaf with worn gaskets; [c] – dried leaf (rehydrated) with worn gaskets, the values of the first line were obtained when the leaf was wet, while the values of the second line were obtained after the leaf was dried; [d] – thermally killed leaf with new gaskets.

Instrument		Chamber area [cm^2]	Plant		
-A			Potato[a]	Potato[b]	Potato[c]
	K_w [µmol s^{-1}]	6	0.72 ± 0.03	1.11 ± 0.05	
			1.06 ± 0.03	1.84 ± 0.14	
			1.60 ± 0.05	2.71 ± 0.04	
	K_c [µmol s^{-1})	6	0.82 (0.9592)	0.96 (0.9724)	0.92 (0.9702)
					0.88 (0.9644)
-B			Potato[a]	Soybean[a]	Potato[d]
	K_w [µmol s^{-1}]	2	0.44 ± 0.00	0.52 ± 0.03	
			0.73 ± 0.08	0.62 ± 0.07	
			1.14 ± 0.03	1.52 ± 0.02	
		6	1.49 ± 0.03	0.39 ± 0.03	0.75 ± 0.03
			2.36 ± 0.47	1.45 ± 0.01	1.06 ± 0.09
			3.38 ± 0.24	2.24 ± 0.06	1.76 ± 0.09
	K_c [µmol s^{-1}]	2	0.43 (0.9273)	0.49 (0.9085)	
		6	0.89 (0.9587)	0.58 (0.9794)	0.56 (0.9047)

initially in a more negative E_A and a higher K_W. The K_W then decreased to a relatively constant value of 2.0 µmol s^{-1} after about 50 min (Fig. 2*A*) (Note that relative humidity was less than 75% to avoid condensing of water within the interior of the air sample lines). Fig. 2*B* showed the changes in calculated K_W of a thermally killed leaf with time. In contrast with the dried leaf results, the water vapor that evaporated from the wet leaf inside the leaf chamber made a larger positive E_A, as this evaporated water vapor was much larger than leakage to the exterior chamber at beginning of the measurement. This larger positive E_A made a negative K_W. As evaporation decreased with time the leaf was balanced with surrounding air (after about 20 min), K_W became positive and stabilized at *ca*. 1.0 µmol s^{-1}.

Table 2 showed K_W and K_C with a dead leaf determined by instrument-A or -B under different conditions. Overall, K_W was consistent for a given leaf. However, it varied between species and amongst individual leaves of the same species in different chambers with different gasket conditions. The range of K_W of the same species was large. For example, for soybean leaves, K_W ranged from 0.39 to 2.24 µmol s^{-1} for the 6 cm^2 chamber with worn gaskets. The K_W with a dead leaf was much less than that of the empty chamber.

K_C was estimated by Eq. 3 as the linear regression slope of $100\,P_{\mathrm{NA}}\,\mathrm{S}-\frac{\mathrm{K_W}\,(\mathrm{W_s}-\mathrm{W_a})\,C_\mathrm{s}}{1000-\mathrm{W}}$ *vs*. $(C_\mathrm{a}-C_\mathrm{s})$.

Overall K_C was stable for different leaves from the same species in a specific chamber as shown in Table 2 for the dead leaves determined by both instrument-A or -B with differently sized chamber and different gaskets. In general, K_C of 2 cm^2 chamber was less than that of 6 cm^2 chamber and K_C with a dead leaf was similar or greater than that for an empty chamber. As expected, K_C

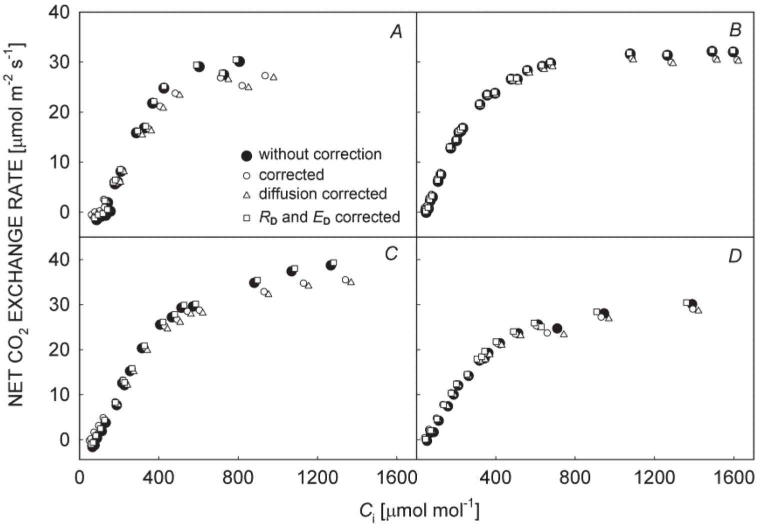

Fig. 3. The net CO_2 exchange rate *vs*. intercellular CO_2 mole fraction (C_i) for potato (*A, B*) and soybean (*C, D*) leaves without correction (*closed circles*), with corrections for both diffusion leakages and inward gas exchanges from leaf under gasket (*open circles*), corrected for only diffusion leakages (*open triangles*), and corrected for only inward gas exchanges from leaf under gasket (*open squares*). The measurements were conducted with the *Li-6400-40* leaf chamber (2 cm^2) (*A, C*) and *Li-6400 2B* leaf chamber (6 cm^2) (*B, D*).

Table 3. The parameters of Farquhar *et al.* (1980) model estimated by P_N/C_i curves, corrected with both the leakages and inward gas exchanges under the gasket, without correction, corrected with only the leakages, and corrected with only inward gas exchange from the leaf under the gasket, for two leaf chamber sizes and for potato and soybean leaves. [a] – the value was corrected for both diffusion leakages and inward gas exchange from the leaf portion under the gasket; [b] – the value was corrected for only the diffusion leakages; [c] – the value was corrected for only the inward gas exchange from the leaf portion under the gasket; [d] – CV is the corrected value.

Plant	Chamber	Parameter	Corrected	Without correction		Diffusion correction[b]		Inward dark[c]	
	size [cm^2]	[µmol m^{-2} s^{-1}]	value[a]	value	value/CV[d]	value	value/CV	value	value/CV
potato	2	V_{cmax}	80.39	87.54	1.09	72.67	0.90	88.90	1.11
		J_{max}	100.10	114.31	1.14	100.85	1.01	108.38	1.08
		TPU	7.22	9.07	1.26	7.52	1.04	8.69	1.20
		R_L	1.76	4.73	2.69	2.68	1.52	3.62	2.06
	6	V_{cmax}	129.20	141.01	1.09	126.23	0.98	143.77	1.11
		J_{max}	121.53	134.74	1.11	124.23	1.02	131.78	1.08
		TPU	8.54	9.66	1.13	8.74	1.02	9.46	1.11
		R_L	0.10	2.38	23.78	0.87	8.68	1.61	16.04
soybean	2	V_{cmax}	95.11	105.01	1.10	90.93	0.96	110.93	1.17
		J_{max}	117.64	141.96	1.21	122.10	1.04	138.18	1.17
		TPU	8.98	10.97	1.22	9.34	1.04	10.63	1.18
		R_L	0.91	4.59	5.04	2.35	2.58	3.22	3.53
	6	V_{cmax}	123.46	101.91	0.83	89.69	0.73	140.79	1.14
		J_{max}	106.44	119.12	1.12	106.91	1.00	118.69	1.12
		TPU	7.93	8.89	1.12	8.06	1.02	8.63	1.09
		R_L	0.00	2.06		0.71		0.95	

of 0.56 µmol s^{-1} for 6 cm^2 chamber with new gaskets filled with a thermally killed potato leaf was less than that of 0.89 µmol s^{-1} with worn gaskets (Table 2), implying that the worn gasket should be replaced regularly to get less leakage error.

We used instrument-A to compare the effect of leaf structure on K_C for a 6 cm^2 chamber as K_C was determined with dried leaves, thermally killed leaves and a rehydrated leaf. The results showed that K_C of dried leaves was greater than that of the rehydrated leaf which was greater than that of thermally killed leaves (Table 2), and the K_C difference between dried leaves and thermally killed leaves was 17%, implying that the structure of a dead potato leaf could influence K_C. Considering the structural similarity of a dead leaf to a living leaf, the correction of measurement error should be based on the value estimated by a thermally killed leaf. Even K_C in the 2 cm^2 chamber could be up to two times less than that in the 6 cm^2 chamber with thermally killed leaves for both potato and soybean plants; however, the 2 cm^2 chamber will have larger leakage errors than 6 cm^2 chamber since the leakage error is based on unit area (Eq. A31a).

Effects on photosynthesis parameterization from P_N/C_i curves: To correct the effects of inward gas exchange (R_D and E_D) from the leaf under gasket on measurements, R_D and E_D of a leaf have to be determined. Using instrument-B with the 6 cm^2 chamber, R_{DA} and E_{DA} were measured with three leaves for each potato and soybean plant. After applying Eq. A24 and Eq. A25, R_D was

0.40 ± 0.10 µmol m^{-2} s^{-1} and E_D was 0.08 ± 0.10 mmol m^{-2} s^{-1} for potato leaves. R_D was 0.67 ± 0.32 µmol m^{-2} s^{-1} and E_D was 0.14 ± 0.05 mmol m^{-2} s^{-1} for soybean leaves. The large variation of R_D or E_D reflected differences among leaves from the same plant, which may reflect varying leaf physiological condition.

The effects of measurement errors from the L_W and L_C, and the E_D and R_D on P_N/C_i curves were examined using two chamber sizes and two different plants (Fig. 3). Overall, P_N corrected for E_D and R_D, and L_W and L_C, was under-estimated at low C_i and over-estimated at high C_i, which was mainly because of the change in the direction of the CO_2 gradient. C_i was larger than that estimated by the *Li-6400* before correction. However, if P_N and C_i were only corrected for E_D and R_D they were closer to the values observed without correction. The measurement errors in the 6 cm^2 chamber were less than that in 2 cm^2 chamber. For example, in 2 cm^2 chamber, P_N was 30.4 µmol m^{-2} s^{-1} corresponding to 32.0 µmol m^{-2} s^{-1} without correction, an overestimation of 1.6 µmol m^{-2} s^{-1} (Fig. 3*C*); in 6 cm^2 chamber, the measurement error was only 1.1 µmol m^{-2} s^{-1} (Fig. 3*D*). The relative measurement error was larger at low C_i. For example, for soybean leaf in 2 cm^2 chamber, at C_i 40 µmol mol^{-1}, the relative measurement error could be 176% but at a high C_i of 1,400 µmol mol^{-1}, the relative error was only about 5% (Fig. 3*C*).

V_{cmax}, J_{max}, TPU, and R_L parameters were estimated with and without correction for only E_D and R_D, only L_W and L_C, and for all these measurement errors from the P_N/C_i curves for potato and soybean leaves (Table 3). R_L was the most sensitive to measurement errors since the R_L was relative small. For example, R_L of a soybean leaf determined by the 2 cm^2 chamber was 4.95 µmol m^{-2} s^{-1} which overestimated the corrected value by five times (Table 3). J_{max} and TPU were overestimated but V_{cmax} could be overestimated or underestimated since both C_i and P_N were affected not only in magnitude but also by the direction of H_2O and CO_2 gradients. If only corrected by diffusion leakages, V_{cmax} typically declined but J_{max} and TPU were enhanced. When only the effects of E_D and R_D were included, all parameters increased for soybean leaf in the 6 cm^2 chamber. Because E_D and R_D were assumed as constant values while effects of diffusion leakages could change direction according the gradient, only taking account for a single effect might lead to bigger measurement errors than without correction, suggesting that the appropriate method must be applied.

Discussion

Leakage coefficients of the empty chamber: The equations for estimating K_W and K_C (Eqs. 1, 2) were developed for an empty chamber based on mass balance equations and the DGM for diffusions of a ternary gas mixture (H_2O, CO_2, and dry air). The calculations of K_W and K_C are independent of each other due to approximations in the equation derivations, *i.e.* CO_2 leakage and concentration are very small compared to the leakage and concentration of H_2O (or dry air), respectively. Eq. A14 is the same as suggested by the manufacturer (*Li-Cor* 2008). The manufacture's equation is based on Fick's law. As noted earlier, Fick's law is not theoretically correct for a ternary gas mixture and does not include Knudsen diffusion. As such, the leakage and measurement error correction equations in previous studies (*Li-Cor* 2008, Rodeghiero *et al*. 2007, Flexas *et al*. 2007) do not include the dry air component. In addition, there is no flow correction term needed in our expressions for determining K_W and K_C (Eqs. A15, A16), *i.e.* L_W and L_C do not alter the bulk flow rate though the leaf chamber since they are balanced by L_A. Rodeghiero *et al.* (2007) incorrectly adapted the equations of von Caemmerer and Farquhar (1981), used to describe H_2O and CO_2 exchanges through a stoma with no dry air source/sink, to estimate K_W and K_C, and included a flow correction term. According to the authors, this resulted in *ca*. 2% higher values of K_W, while the effect of their flow correction on K_C estimates could be 20–60% for the chamber CO_2 mole fractions ranging from 1,200–2,000 µmol mol^{-1}.

In the present study, K_C for the 6 cm^2 and 2 cm^2 chambers (Fig. 1*C,D*) of instrument-B with the worn gaskets were similar to the values of 0.46, 0.40, and 0.44 µmol s^{-1} from the *Li-6400* manual (*Li-Cor* 2008), Rodeghiero *et al.* (2007), and Flexas *et al.* (2007) respectively. However, it was greater than the value of cca. 0.2 µmol s^{-1} estimated by McDermitt *et al*. (2001) and less than the results from instrument-A. The leakage of H_2O has been little considered (Rodeghiero *et al.* 2007). Water leakage is not only an issue in the transpiration measurement, but also a potential problem in calculations of K_C (Eq. 3), P_N (Eq. 5), g_s (Eq. 6) and C_i (Eq. 7), and, in fact, K_W was 8 to 10-fold greater than K_C (Fig. 1). Using the same equation, for the 6 cm^2 chamber, K_W was much higher than the value 0.89 µmol s^{-1} determined by Rodeghiero *et al*. (2007), and much lower than the value 6.78 µmol s^{-1} for the 2 cm^2 chamber or the value determined in the instrument-A.

Consistent linear relationships as indicated in Eq. 1 and Eq. 2 for K_W and K_C and Fig. 1 were also found in reinterpretation of the results from Flexas *et al.* (2007) for K_C and Rodeghiero *et al*. (2007) for both K_W and K_C. We concluded that the models of equations (1) or (2) are valid for estimating K_W and K_C for an empty chamber, and the different values of K_W and K_C in instruments A and B may reflect a difference in instrument conditions, such as leaf gasket tightness, gasket width and age, and condition of the O ring and back gasket seal. These results strongly suggested that K_W and K_C should be determined for each specific instrument (and chamber).

Effect of presence of a leaf: K_W and K_C of the chamber with a dead leaf (dried or thermally killed) were different from those of an empty chamber since the leakages between leaf and gasket are different from leakages between gaskets. Because both types of dead leaves can be a H_2O source/sink, the determination of K_W (Eq. 1) for a chamber filled with a dead leaf should be under the condition that leaf water is balanced with air inside the leaf chamber. When the dead leaf is placed into a new environment, a new water balance is established with the surrounding air, *i.e.* from a H_2O source/sink to a neutral conditions. At this stage, K_W can be obtained but the process for a dried or thermally killed leaf may take 20 min or longer (Fig. 2).

In the 2 cm^2 chamber, K_W for a dried potato or soybean leaf (Table 2) was much lower than the value of 5.11 µmol s^{-1} estimated by Rodeghiero *et al.* (2007) for a dead *Quercus ilex* leaf, while in 6 cm^2 chamber, the average value of K_W was similar in comparison with the value 1.86 µmol s^{-1} of Rodeghiero *et al.* (2007). While the present study observed small K_W values regardless of chamber size (Table 2), Rodeghiero *et al.* (2007), showed a larger difference between two sized chambers. They explained the reason was partly due to effects of water vapor sorption and desorption by the dried leaf for the K_W determination. Indeed, their leaf chamber was moister than the outside air, as a result their K_W would be larger than that at water balance state (Fig. 2*A*), and a small chamber usually needs a longer time period to reach a steady state (*Li-Cor* 2008). The relative large variations of K_W might reflect the more sensitive response of K_W to the different structure of the dead leaves.

The determination of K_C using a dried leaf was recommended by Long and Bernacchi (2003), and analyzed by Rodeghiero (2007) with *Quercus ilex* L. leaves; however, Flexas *et al.* (2007) argued that characteristics of a thermally killed leaf more closely resembled those of a living leaf. Our equation for determining K_C (Eq. 3) with a thermally killed leaf differed from the method of Flexas *et al.* (2007) and Alonso *et al.* (2009). Eq. 3 can be rearranged as:

$$P_{NA} = \frac{S\,K_C}{100}\,C_s + \left[\frac{K_W\,(W_s - W_a)\,C_s}{100\,S\,(1000 - W_s)} - \frac{S\,K_C\,C_a}{100}\right] \qquad (8)$$

To obtain the linear relationship between P_{NA} and C_s based on the slope of S K_C/100, the authors were restricted to the special case where the second term was small [*i.e.* ($W_S - W_A$) C_s is small] and C_a was constant (Flexas *et al.* 2007, Alonso *et al.* 2009). These conditions are restricted to conditions with small water vapor leakage and stable values of ambient CO_2 concentration, whereas such restrictions do not apply for Eq. 3 in the current study. Our method also differed from Rodeghiero *et al.* (2007) who determined Kc with a dried leaf using a variation of Eq. A22a when E_A is very small. The authors obtained K_C as the slope of the linear relationship in $\frac{100\,(C_s - C_r)}{C_a - C_s}$ *vs.* $1/F_i$, but only when C_r is small (Rodeghiero *et al.* 2007 used a value of 50 µmol mol^{-1}). The significant linear regressions of $100\,P_{NA}\,S - \frac{K_W\,(W_s - W_a)\,C_s}{1000 - W_s}$ *vs.* $C_a - C_s$ in our experiments (Table 2), and of P_{NA} *vs.* C_s in Alonso *et al.* (2009) and Flexas *et al.* (2007) suggest estimates for K_C with thermally killed leaves were correct, and variation in K_C values with different leaves were presumably due to differences in leaf structure.

K_C measured by instrument-B in the 2 cm^2 chamber with a thermally killed leaf was similar to the empty chamber, but larger than that with a tobacco leaf (Flexas *et al.* 2007), and a spring wheat leaf (Alonso *et al.* 2009) (using a different gas analyzer: *CIRAS-2, PP System*, Hitchin, Hrtyd, UK with a 1.7 cm^2 chamber). The 2 cm^2 instrument-B K_C value was also lower than the one estimated in instrument-A and -B fitted with the 6 cm^2 chamber. Overall, such comparisons along with the results in Table 2 indicated that the equation we developed was reliable. Observed differences in K_C for leaves from different plant species could be explained as a result of different leaf structures and instrument bias. As K_C within a given instrument and chamber size varied with leaf condition (dried or thermally killed) and whether or not the chamber was empty, we suggest that K_C be determined with a thermally killed leaf to correct for measurement errors, in light of the fact that the leaf structure is most similar to a living leaf (Flexas *et al.,* 2007). The lower K_C for new versus worn gaskets (0.56 *vs.* 0.89 µmol s^{-1}) contrasted with Rodeghiero *et al.* (2007), who found K_C for worn gaskets was 20–30% lower than the K_C for new gaskets. This difference further suggests that K_C be determined, and the correction be made, under similar gasket conditions to decrease correction errors due to different gasket conditions.

The correction of measurement error: According to Eq. 4 and Eq. 5, any method that can (*1*) reduce H_2O and CO_2 mole fraction gradients between inside and outside chamber will decrease leakage errors, (*2*) decrease permeability for CO_2 and H_2O between gasket and leaf can also decrease the leakage errors since the major leakages are between gasket and leaf rather than the gasket itself, and (*3*) increase the chamber window area and/or reduce the gasket area can reduce leakage and dark transpiration and respiration errors. However, in practice, one method may be offset by another, *i.e.* reducing the gasket area to decrease the inward gas exchange released from the leaf portion under the gasket may increase the permeability or *vice versa*. Several methods to minimize leakage of CO_2 were tested by Flexas *et al.* (2007), but leakage of H_2O, and inward E_D and R_D released from the leaf under the gasket were not considered. They argued that methods to reduce chamber leakage did not seem promising for correcting measurements. Rodeghiero *et al.*

(2007) suggested using a double gasket design to reduce the diffusion leakages, thereby diminishing gas gradients. In the present study, since both K_C and K_W were easy to estimate and the values were very stable for a specific leaf and instrument, we strongly recommend that measurement errors be corrected by using K_C and K_W, and E_D and R_D under the specific experimental conditions desired by the user.

Flexas *et al.* (2007) and Alonso *et al.* (2009) corrected net CO_2 exchange rate by simple subtraction of the relationship between C_s and P_{NA} of a chamber filled with a dead leaf. Alonso *et al.* (2009) suggested that H_2O diffusion was too small to need to correct for. As indicated in Eq. 8, their method is a good approximation for correcting P_{NA}, but only if the effect of dark respiration from the leaf under the gasket can be ignored and the same values of $(W_s – W_a)$ C_s and ambient CO_2 are maintained when determining the CO_2 leakage and measuring P_N for a living leaf (Eqs. 5, 8). The correction methods for P_N suggested by Rodeghiero *et al.* (2007) and the manufacture (*Li-Cor* 2008) also did not include both effects of R_D and H_2O leakage. The method provided by Pons and Welschen (2002) only considered the measurement error due to respiration under the gasket (*see* also Lenz *et al*. 2010). The correction method for *E* suggested by Rodeghiero *et al.* (2007) simply added the H_2O leakage and did not correct for the correction of flow rate, which is typically a 3% error; it also did not account for the dark transpiration from the leaf under the gasket. Our suggested approaches address these limitations.

Effects on parameterization of model of Farquhar: The parameterization of the Farquhar *et al.* (1980) biochemical model for a C_3 leaf was based on P_N/C_i curves using methodology in Sharkey *et al.* (2007). C_i was calculated by Eq. 7a for potato, or by Eq. 7b for the soybean leaf. Thus, effects of all measurement errors involved in correcting P_N and C_i on the parameterization were evaluated. Note the results in Fig. 3 and Table 3 were examples for specific potato and soybean leaves under specific conditions, *i.e.* the range of water vapor gradient was typically between –15 to +15 mmol mol^{-1} and it was assumed constant values of the R_D and E_D. If experimental conditions change, the magnitude of the effects of measurement errors will alter accordingly.

The effects of diffusion leakages on C_i are mainly dependent on the correction of P_N due to L_C (Eqs. 7a, 7b). For example, for the P_N/C_i measurement using the 2 cm^2 chamber for the potato leaf, at a C_s of 1,963 µmol mol^{-1} and water vapor gradient of 8.2 mmol mol^{-1}, the C_i was underestimated by 28 µmol mol^{-1} if only considering L_W. However, C_i would be underestimated by 109 µmol mol^{-1} if L_C was included. The effect of L_W was less important than the effect of L_C, but was significant. This contrasts with the conclusion that H_2O diffusion leakages influence P_N/C_i curves at least as much as CO_2 diffusion leaks (Rodeghiero *et al.* 2007).

Fig. 3 and Table 3 show effects of correcting for different measurement errors on the P_N/C_i curves and model parameters. The significant differences between corrections for all experimental errors as opposed to only diffusive leakages and inward R_D and E_D suggest that correcting for only a portion of these errors might be worse than making no corrections at all since the magnitude and direction of L_W and L_C change according to their gradients, while the direction and effects of inward R_D and E_D from under gasket do not change. All previous studies only corrected for a portion of the measurement errors, such as the error due to R_D under the gasket (Pons and Welschen 2002, Lenz *et al*. 2010), only the CO_2 leakage (*e.g.*, Flexas *et al.* 2007), or only H_2O and CO_2 leakages (*e.g.*, Rodeghiero *et al*. 2007).

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Appendix: Detailed Equation Derivations

(*1*) Diffusive leakage coefficients of an empty chamber

At steady-state condition, the mass balance equations for H_2O and CO_2 with diffusion leakages into an empty chamber can be expressed as

$$\frac{F_o\,W_s - F_i\,W_r}{10^6} = L_W \tag{A1}$$

and

$$\frac{F_o\,C_s - F_i\,C_r}{10^6} = L_C \tag{A2}$$

where F_i and F_o are the incoming and outgoing air flow rate, respectively; W_s and W_r are the sample and reference water vapor mole fraction, C_r and C_s are the sample and reference CO_2 mole fraction; L_W and L_C are the water and CO_2 leakage rate.

At steady-state condition, the air mixture pressure in an empty chamber keeps constant. Based on Dalton's law and the ideal gas law, the total air mixture pressure in the leaf chamber can be expressed as

$$P_{am}\,V = (n_{CO2} + n_{H2O} + n_{da})\,R\,T \tag{A3}$$

where P_{am} is the pressure of the air mixture inside the chamber; V is the volume of the chamber, R is the universal gas constant, T is the temperature of the leaf chamber; and the n_{CO2}, n_{H2O} and n_{da} are the number of moles of CO_2, of H_2O and dry air respectively. Because V, R and T keep constant, the total number of moles of n_{CO2}, n_{H2O} and n_{da} must be a constant. Therefore, the total leakages of CO_2, H_2O and dry air (L_W) is equal to zero.

$$L_C + L_W + L_A = 0 \tag{A4}$$

Because of the H_2O, CO_2 diffusive leakages, $W_s \neq W_r$ and $C_s \neq C_r$ in Eq. A1 and Eq. A2; however, since the sum of leakages of H_2O, CO_2 and dry air is zero (Eq. A4), $F_o = F_i$. Eq. A1 and Eq. A2 can be simplified

$$L_W = \frac{F_i\,(W_s - W_r)}{10^6} \tag{A5}$$

and

$$L_C = \frac{F_i\,(C_s - C_r)}{10^6} \tag{A6}$$

The air mixture can be treated as ternary system with H_2O, CO_2 and dry air (Jarman 1974, von Gaemmerer and Farquhar 1981, Leuning 1983). The DGM (Mason and Malinauskas 1983) can describe the leakages of H_2O and CO_2 through the leaf chamber. Ignoring both thermal and baric diffusions, which are typically small, the H_2O and CO_2 leakages can be approximately obtained by

$$\frac{\Delta W}{10^6\,\Delta l} = -\frac{\overline{A_{as}}\,L_W - \overline{W_{as}}\,L_A}{S\,C_{AM}\,D_{wa}} - \frac{\overline{C_{as}}\,L_W - \overline{W_{as}}\,L_C}{1000\,S\,C_{AM}\,D_{cw}} - \frac{L_W}{S\,C_{AM}\,D_W^k} \qquad \text{(A7)}$$

and

$$\frac{\Delta C}{10^6\,\Delta l} = -\frac{\overline{A_{as}}\,L_C - \overline{C_{as}}\,L_A}{S\,C_{AM}\,D_{ac}} - \frac{\overline{W_{as}}\,L_C - \overline{C_{as}}\,L_W}{S\,C_{AM}\,D_{cw}} - \frac{L_C}{S\,C_{AM}\,D_C^k} \qquad \text{(A8)}$$

where W and C are water and CO_2 concentrations, respectively; Δl is the diffusion effective length; D_{wa}, D_{cw} and D_{ac} are the diffusivities for binary mixtures of H_2O and dry air, CO_2 and H_2O, and air and CO_2, respectively; D_W^k and D_C^k are the Knudsen diffusivities for H_2O and CO_2, respectively; C_{AM} is the total air mixture concentration, which is assumed as a constant; S is the area of the chamber window; $\overline{A_{as}}$, $\overline{W_{as}}$ and $\overline{C_{as}}$ are the mean molar fractions of dry air, H_2O and CO_2 along the leaf chamber, respectively, *i.e.* $\overline{W_{as}} = (W_a + W_s)/2$, $\overline{C_{as}} = (C_a + C_s)/2$, and subscripts a and s indicate ambient and inside leaf chamber, respectively. The first four terms in Eq. A7 and Eq. A8 are due to the collisions of molecules and are based on the Stefan-Maxwell equation. The fifth terms are due to the collisions between gas molecules and pore walls.

Since $D_{ac} \approx D_{cw}$, $D_{wa} \approx 1.6D_{cw}$, the CO_2 mole fraction is a small part of the total gas mixture ($\overline{C_{as}}/10^3 \le \overline{A_{as}} + \overline{W_{as}}$) and CO_2 diffusive leakage rate is small compared to the diffusive leakage of H_2O and dry air ($L_C/10^3 \ll L_W$); $\frac{\overline{A_{as}}}{10^3} + \frac{\overline{W_{as}}}{10^3} + \frac{1.6\,\overline{C_{as}}}{10^6} \approx \frac{\overline{A_{as}}}{10^3} + \frac{\overline{W_{as}}}{10^3} \approx 1$, $L_C + L_W = -L_A \approx L_W$ and the fourth term in Eq. A7 is negligible. We obtain

$$\frac{\Delta W}{10^6\,\Delta l} = -\left(\frac{1}{S\,C_{AM}\,D_{wa}} + \frac{1}{S\,C_{AM}\,D_W^k}\right) L_W \qquad \text{(A9)}$$

and

$$\frac{\Delta C}{10^6\,\Delta l} = -\left(\frac{1}{S\,C_{AM}\,D_{ac}} + \frac{1}{S\,C_{AM}\,D_C^k}\right) L_C \qquad \text{(A10)}$$

The diffusive leakage coefficients of H_2O in the air (K_W) and CO_2 in air (K_C) are defined as

$$\frac{1}{K_W} = \frac{\Delta l}{S\,C_{AM}}\left(\frac{1}{D_{wa}} + \frac{1}{D_W^k}\right) \qquad \text{(A11)}$$

and

$$\frac{1}{K_C} = \frac{\Delta l}{S\,C_{AM}}\left(\frac{1}{D_{ca}} + \frac{1}{D_C^k}\right) \qquad \text{(A12)}$$

Eq. A9 and Eq. A10 can be simplified to

$$\frac{W_s - W_a}{10^6} = \frac{L_W}{K_W} \qquad \text{(A13)}$$

and

$$\frac{C_a - C_s}{10^6} = -\frac{L_C}{K_C} \qquad \text{(A14)}$$

Eq. A13 and Eq. 14 have the same form as Fick's law, but they are derived from the DGM under the conditions noted above. Combing Eq. A5 and Eq. A13, Eq. A6 and Eq. A14, respectively; we can obtain the working equations for K_W and K_C as

$$K_W = \frac{F_i\,(W_s - W_r)}{(W_a - W_s)} \qquad \text{(A15)}$$

and

$$K_C = \frac{F_i\,(C_r - C_s)}{(C_s - C_a)} \qquad \text{(A16)}$$

Note that Eq. A15 is different from Eq. 3 of Rodeghiero *et al.* (2007). There is no flow rate correction in Eq. A15 for an empty chamber. Eq. A16 is different from Eq. 6 of Rodeghiero *et al.* (2007). Leakage of H_2O does not affect K_C for an empty chamber.

(*2*) Leakage coefficients of the chamber with a dead leaf

The K_W and K_C might be significantly different when a living leaf is enclosed in the chamber *vs*. an empty chamber because the leakage between gasket and leaf may be different from the leakage between gaskets. The leakage coefficients should be determined under experimental conditions with as close to a real living leaf as possible. As suggested by Flexas *et al.* (2007), a thermally killed leaf, or a dried leaf as used by Long and Bermacchi (2003) could be considered as a substitute for a living leaf for this purpose. However, because the dead leaf can be a source/sink of H_2O, the K_W cannot be determined directly by Eq. A15, and the K_C cannot be calculated using Eq. A16. At steady state, the net flux of a gas can be closely approximated by the sum of independent contributions of diffusive flow. When there is a leaf inside a chamber, mass balance for H_2O can be expressed as

$$\frac{\mathrm{F_o\,W_s - F_i\,W_r}}{10^6} = 10^{-4}\,\mathrm{S}\,E - \mathrm{L_W} + 10^{-4}\,\mathrm{S\,D_W} \tag{A17}$$

where D_W is the evaporation escape from the leaf under gasket. The mass balance of gas mixture is given as (ignoring the change in CO_2)

$$\mathrm{F_o} = \mathrm{F_i} + 0.1\,\mathrm{S}\,E - 1000\,\mathrm{L_W} - 1000\,\mathrm{L_A} + 0.1\,\mathrm{S\,D_W} \tag{A18}$$

Since $\mathrm{L_W} \approx -\mathrm{L_A}$ (Eq. A4), Eq. A18 can be simplified to

$$\mathrm{F_o} = \mathrm{F_i} + 0.1\,\mathrm{S}\,E + 0.1\,\mathrm{S\,D_W} \tag{A19}$$

Substituting Eq. A19 into Eq. A17,

$$E = \frac{10\,\mathrm{F_i\,(W_s - W_r)}}{\mathrm{S\,(1000 - W_s)}} + \frac{10^7\,\mathrm{L_W}}{\mathrm{S\,(1000 - W_s)}} - \mathrm{D_W} \tag{A20a}$$

The first term of Eq. A20a is the apparent transpiration rate (E_A). The second term is the influence of L_W modified by the effect of H_2O dilution inside the chamber. Substituting Eq. A13 to Eq. A20a, we have

$$E = E_\mathrm{A} + \frac{10\,\mathrm{K_W\,(W_s - W_a)}}{\mathrm{S\,(1000 - W_s)}} - \mathrm{D_W} \tag{A20b}$$

If water from the dead leaf evaporates, it is not possible to determine E prior to determination of K_W. However, if W_r keeps stable, the water vapor of dead leaf will be eventually balanced with W_s, *i.e.*, $E = 0 = D_W$. Under these conditions, Eq. A20 becomes Eq. A15. Note that estimating K_W in the chamber with a dead leaf requires two conditions: one is steady state and another $E = D_W = 0$. When approaching this point, the thermally killed leaf becomes a dried leaf. A very dried leaf may become a little wet by absorbing water vapor from its environment. Therefore, K_W estimated from a dead leaf (thermally killed leaf or dried leaf) represents its value when the dead leaf balanced with it environment. The working equation for leakage coefficient of water vapor has the same form as for an empty chamber (Eq. A15).

Since there is no CO_2 source/sink inside the chamber filled with a dead leaf, the mass balance of CO_2 can be given by Eq. A2. Substituting Eq. A19 into Eq. A2, we obtain

$$\frac{\mathrm{F_i}\,(C_\mathrm{r} - C_\mathrm{s})}{100\,\mathrm{S}} - \frac{C_\mathrm{s}\,(E + \mathrm{D_W})}{1000} = \frac{\mathrm{L_C}}{10^{-4}\,\mathrm{S}} \tag{A21}$$

Substituting Eq. A20b into Eq. A21,

$$\frac{\mathrm{F_i}\,(C_\mathrm{r} - C_\mathrm{s})}{100\,\mathrm{S}} - \frac{E_\mathrm{A}\,C_\mathrm{s}}{1000} - \frac{\mathrm{K_W\,(W_s - W_a)}\,C_\mathrm{s}}{100\,\mathrm{S\,(1000 - W_s)}} = \frac{\mathrm{L_C}}{10^{-4}\mathrm{S}} \tag{A22a}$$

The first two terms of Eq. A22a is the P_{NA}. The third term is the influence of H_2O leakage. Eq. A22a becomes,

$$P_{\mathrm{NA}nA} - \frac{\mathrm{K_W\,(W_s - W_a)}C_\mathrm{s}}{100\,\mathrm{S\,(1000 - W_s)}} = \frac{\mathrm{K_C}\,(C_\mathrm{s} - C_\mathrm{a})}{100\,\mathrm{S}} \tag{A22b}$$

Eq. A22b can be rearranged as

$$\mathrm{K_C} = \frac{100\,P_\mathrm{NA}\,\mathrm{S} - \frac{\mathrm{K_W\,(W_s - W_a)}\,C_\mathrm{s}}{1000 - \mathrm{W}}}{C_\mathrm{s} - C_\mathrm{a}} \tag{A23a}$$

Note that Eq. A23a is different from Eq. 6 of Rodeghiero *et al.* (2007). It also includes the effect of leaf evaporation (in P_{NA}). When the second term can be negligible compared to the first term (*e.g.* when W_a is close to W_S and/or C_s is very small), Eq. A23a can be simplified as

$$K_C = \frac{100\, P_{NA}\, S}{C_s - C_a} \qquad \text{(A23b)}$$

(*3*) Inward gas exchanges and correction for the measurement errors for gas leakages

When the leaf chamber clamps onto a leaf to measure P_N and E, D_E and D_C released by the dark transpiration (E_D) and respiration (R_D) from the leaf under gasket will enter into the interior of the leaf chamber, leading to a higher E_A and a lower P_{NA}. D_C can be estimated by (Pons and Welschen 2002, Lenz *et al.* 2010)

$$D_C = r\, R_D \qquad \text{(A24)}$$

Since H_2O and CO_2 exchanges share the same pathway, D_W is determined by

$$D_W = r\, R_W \qquad \text{(A25)}$$

where r is the ratio of the gasket area of the inward portion to the chamber window area (Pons and Welschen 2002, Shapiro *et al.* 2004, Lenz *et al.* 2010) for homobaric leaves with high resistance to lateral gaseous transport and heterobaric leaves. The ratios of two chambers used in this study are listed in Table 4. The E_D and R_D can be obtained by

$$E_D = \frac{E_{DA} + \frac{10\, K_W\, (W_S - W_a)}{S\,(1000 - W_S)}}{1 + r} \qquad \text{(A26)}$$

and

$$R_D = \frac{R_{DA} + \frac{K_W\, (W_S - W_a)\, C_S}{100\, S\,(1000 - W_S)} - \frac{K_C\, (C_a - C_S)}{100\, S}}{1 + r} \qquad \text{(A27)}$$

At steady state, when there is a living leaf inside a chamber, mass balance for H_2O flux and for mixture gas can be expressed as Eq. A17 and Eq. A19, respectively. The working equation for the correction of transpiration is

$$E = E_A + \frac{10\, K_W\, (W_S - W_a)}{S\,(1000 - W_S)} - r\, E_D \qquad \text{(A28)}$$

Note that Eq. A28 is different from the Eq. 10 of Rodeghiero *et al.* (2007). The effect of leakage of H_2O is adjusted by flow rate change. The mass balance of CO_2 can be given by

$$\frac{F_o\, C_S - F_i\, C_r}{1000} = -10^{-4}\, S\, P_N - L_C - 10^{-4}\, S\, D_C \qquad \text{(A29)}$$

Table 4. The dimensions of two standard leaf chambers of *Li-6400*.

Chamber model	*6400-2B*	*6400-40*
Window area [cm^2]	6.00	2.00
Gasket area [cm^2]	7.44	4.14
Gasket width [cm]	0.60	0.60
Gasket inward part [cm^2]	3.31	1.79
Ratio of gasket inward part to window area	0.55	0.89

Substituting Eq. A19 into Eq. A29, we obtain,

$$P_N = \frac{F_i\, (C_r - C_S)}{100\, S} - \frac{E\, C_S - D_W\, C_S}{1000} - \frac{L_C}{10^{-4}\, S} - D_C \qquad \text{(A30)}$$

Substituting Eq. A20b and Eq. A24 into Eq. A30,

$$P_N = P_{NA} - \frac{K_W\, (W_S - W_a)\, C_S}{100\, S\,(1000 - W_S)} + \frac{K_C\, (C_a - C_S)}{100\, S} - r\, R_D \qquad \text{(A31a)}$$

Note that Eq. A31a is different from Eq. 13 of Rodeghiero *et al.* (2007). It takes effects of adjusted $L_W C_s$ and inward respiration into account. When W_s is close to W_a or C_S is very small, Eq. A31a is simplified to

$$P_{\mathrm{N}} = P_{\mathrm{NA}} + \frac{\mathrm{K_C}\,(C_{\mathrm{a}} - C_{\mathrm{s}})}{100\ \mathrm{S}} + \mathrm{D_C} \qquad \text{(A31b)}$$

(*4*) The measurement protocol:

Carefully select at least 3 leaves with similar structure to living leaves that will be measured. The leaves are killed by immersion in boiling water until no variable chlorophyll fluorescence is detectable as measured by a chlorophyll meter. The thermally killed leaf is then blotted and enclosed in the chamber of a ready to be used open gas-exchange system.

$\mathrm{K_C}$ is determined by performing $P_{\mathrm{NA}}/C_{\mathrm{i}}$ curves at C_{a} of 50, 200, 1,500; and 2,000 µmol mol^{-1} using Eq. 3. The measurements will be completed before the leaf is dry.

$\mathrm{K_W}$ is determined when E_{A} is stable by Eq. 1. A low negative water vapor gradient (lower than 10 mmol mol^{-1}) between inside and outside the leaf chamber can be achieved by adjusting the valve of the desiccant tube to decrease the water vapor mole fraction inside the chamber. A large positive water vapor gradient (larger than 10 mmol mol^{-1}) can be achieved by adding a few drops of distilled water to the soda lime tube to increase water vapor mole fraction inside the chamber.

Dark transpiration is determined by Eq. A26 and dark respiration by Eq. A27. At least 3 leaves with similar structure and age to living leaves will be measured are selected.

Correction of measurement errors by Eq. 4 and Eq. 5 and calculation g_{s} and C_{i} by Eq. 6 and Eq. 7. The ambient mole fractions of CO_2 and H_2O should be recorded. In practice, there may not be CO_2 and H_2O sensors available to monitor C_{a} and $\mathrm{W_a}$. Ambient CO_2 and H_2O can be obtained by the same open gas exchange system before and after each measurement or each $P_{\mathrm{N}}/C_{\mathrm{i}}$ curve.