

Are measurements from excised leaves suitable for modeling diurnal patterns of gas exchange of intact leaves?

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Abstract

To examine the feasibility of using ecophysiological data from excised leaves for a meteorological simulation model of gas exchange, we compared the obtained gas exchange rates and the modelled ones using excised leaves and those using intact leaves. Instantaneous gas exchange rates of excised leaves and stomatal control in response to environmental conditions were not significantly different from those of attached leaves. Modelled gas exchange rates based on excised leaf data showed a good fit to the diurnal patterns of *in situ* measurements. This suggests that use of excised leaf data to predict gas exchange of intact leaves is permissible as long as the effects of excision are pre-screened as described in this paper. Copyright © 2011 John Wiley & Sons, Ltd.

Key Words ecophysiological model; excised leaf; gas exchange; *in situ* measurement; intact leaf; stomatal control

Introduction

In forests, environmental factors vary temporally, creating diurnal patterns of leaf gas exchange (transpiration and photosynthesis). Trees also spontaneously modify gas exchange by stomatal control in response to the environment (Ball *et al.*, 1987; Leuning, 1995). To reproduce the diurnal patterns of gas exchange, many studies have established ecophysiological models (Harley *et al.*, 1992; Leuning, 1995; Lloyd *et al.*, 1995; Wang and Leuning, 1998). Using data from intact leaves, models successfully reproduced leaf- and stand-level gas exchanges, which were checked with *in situ* measurement using chamber or eddy covariance methods (Harley and Baldocchi, 1995; Leuning *et al.*, 1995; Baldocchi and Meyers, 1998; Lai *et al.*, 2000; Kumagai *et al.*, 2006).

Data from intact leaves of trees are, however, available only for trees that are within reach of scaffold towers or cranes. In research at larger spatial scales (Mackay *et al.*, 2002; Ford *et al.*, 2007), scaffolding towers are not available everywhere. For these studies, a practical method for gas exchange estimation would be to obtain data from canopy leaves excised using a pole pruner (Irvine *et al.*, 1998; Kume *et al.*, 2003). Instantaneous light-saturated photosynthetic rates of excised leaves have been found to be reliable (Reich *et al.*, 1995; Cai *et al.*, 2009; Joesting *et al.*, 2009) after pre-screening the effects of excision. On the other hand, stomatal control and resulting modelled gas exchange are believed to be altered by excision and subsequent change in water conditions (Sala and Tenhunen, 1996; Santiago and Mulkey, 2003; Ewers *et al.*, 2007; Domingues *et al.*, 2010). Therefore, until now, excised leaf data have not been recognized as an alternative to intact leaf data in the meteorological model simulation.

In this study, we measured gas exchange traits of intact leaves and those after excision using mature evergreen broad-leaved trees, *Lithocarpus edulis* Nakai., in mid summer under high atmospheric evaporative demand. Using a well known ecophysiological model (Leuning *et al.*, 1995), diurnal patterns of gas exchange were simulated based on leaf data before and after excision.

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The suitability of model simulations using data of excised leaves was examined by addressing the following questions: (1) Are there consistent differences in gas exchange traits or stomatal control between intact leaves and the same leaves after excision? (2) Do models based on excised leaf data accurately predict the diurnal patterns of leaf gas exchange of intact leaves?

Materials and Methods

Study sites

This study was carried out in the Kasuya Research Forest, Kyushu University (33° 37'N, 130° 31'E, 50 m above sea level.), Fukuoka, Japan. In the 5 years before 2007, the averages of mean air temperature, daily minimum temperature in the coldest month (January), and daily maximum temperature in the hottest month (August) were 16.2, 1.3, and 33.4 °C, respectively. The mean annual precipitation was 1560 mm.

We carried out measurements in a matured plantation of the evergreen broad-leaved tree species, *L. edulis*. The mean height and diameter at chest height were 8 m and 18 cm, respectively. We accessed the top canopy using a wide-breadth scaffolding tower set up within the stand, which enabled us to measure spatial and temporal variation in gas exchange of the intact leaves. Trees of *L. edulis* expand new leaves in a flush in May and shed leaves older than one year soon after new leaf emergence in May. The trees flower in June.

Leaf gas exchange measurements with intact and excised leaves

In June 2009, we selected three individuals for leaf gas exchange measurements within reach from the tower. We selected two well-lit shoots per tree and selected three leaves per shoot as sample leaves ($n = 6$ per tree). The leaf age of the sample leaves were 0 and 1 year old ($n = 13$ and 5 for 0 and 1 year old leaves, respectively). Because gas exchange rates of 0-year and 1-year-old leaves were not significantly different (data not shown), their data were pooled for analysis. Measurements were classified as follows: (1) CO₂ and light response measurements of gas exchange of the same leaves before excision (intact leaf data) and gas measurement under two contrasting ambient environments ('two time-point measurements', shown below); (2) Measurements described in (1) with the same leaves after excision (excised leaf data); and (3) *In situ* diurnal measurement of gas exchange (*in situ* measurement data).

For each intact leaf, we first measured net photosynthetic rates (A , $\mu\text{mol m}^{-2} \text{s}^{-1}$), stomatal conductance of H₂O (g_s , $\text{mol m}^{-2} \text{s}^{-1}$) and transpiration rate (E , $\text{mol m}^{-2} \text{s}^{-1}$) under ambient environmental conditions at two time points of contrasting light and temperature environments: early morning (incident photosynthetic photon

flux density (PPFD) ca. 100–250 $\mu\text{mol m}^{-2} \text{s}^{-1}$), and at midday (PPFD >400 $\mu\text{mol m}^{-2} \text{s}^{-1}$). For each leaf, we carried out this 'two time-point measurement' to obtain gas exchange data under varying environmental conditions for the construction of the stomatal control model (Ball *et al.*, 1987). We used a calibrated Li-6400 portable photosynthesis system (Li-cor, Lincoln, NE, USA) in all gas exchange measurements, under a boundary layer conductance of H₂O 2.84 $\text{mol m}^{-2} \text{s}^{-1}$.

We then measured gas exchange under seven levels of intercellular CO₂ concentrations (c_i , $\mu\text{mol mol}^{-1}$) from 20 to 1000 $\mu\text{mol mol}^{-1}$ (CO₂ response measurements) to obtain the parameters in Farquhar *et al.* (1980): maximum rate of RuBP carboxylation (V_{cmax} , $\mu\text{mol m}^{-2} \text{s}^{-1}$) and electron transport for the regeneration of RuBP (J_{max} , $\mu\text{mol m}^{-2} \text{s}^{-1}$). We calculated the photosynthetic capacity of each individual leaf normalized at a common leaf temperature of 25 °C ($V_{\text{cmax},25}$ and $J_{\text{max},25}$). We used Michaelis-Menten parameters in von Caemmerer *et al.* (1994), their temperature dependencies in Harley *et al.* (1992) and those of V_{cmax} and J_{max} of other evergreen broad-leaved tree species co-occurring in this study site (Miyazawa and Otsuki, 2010).

We measured leaf gas exchange of the sample leaves under 11 levels of light intensity levels (PPFD = 0–2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$) to obtain parameters for the light-photosynthesis relationship (Johnson and Thornley, 1984): initial slope (α) and convexity (θ).

On 28 and 31 July 2009, after *in situ* diurnal measurements of leaf gas exchange (shown below), we excised each shoot and re-cut each one in distilled water early in the morning. We transported the shoot to the laboratory to carry out gas exchange measurements (CO₂ response, light response and two time-point gas exchange measurements) using a similar procedure as done for intact leaves. Ambient light intensity of the excised shoot was controlled at 800–1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ to keep the leaves at a photosynthetically induced condition using a lamp (EYE Multi-Hi-Ace, Iwasaki Electronic Co., Ltd., Tokyo, Japan).

This protocol was designed for sites with short forests with easy canopy access using a pole pruner and those with easy access to high light canopy gaps. These conditions are not available in some study sites, especially in closed and taller tropical rain forests, where leaf sampling requires a shotgun. In this study, however, we intended to examine the feasibility of excised leaf data in the absence of effects by other factors, which may be important for other study sites; damage by sampling using a shotgun, stomatal closure and deactivation of Rubisco due to measurements under dark ambient light conditions in the forest understory.

Diurnal patterns of leaf gas exchange

On 23 July 2009, we monitored the gas exchanges of the sample leaves from predawn through evening. We

measured A , dark respiration rate (R_d , $\mu\text{mol m}^{-2} \text{s}^{-1}$), E and g_s for each sample leaf at each time. We used a cuvette with a transparent window at the top. Ambient atmospheric air was supplied at 500 ml min^{-1} .

Gas exchange of the sample leaves was measured at 1–1.5-h intervals. Each leaf was clamped with the leaf position and direction fixed as they were. We also recorded CO_2 concentration (C_s , $\mu\text{mol mol}^{-1}$) and relative humidity inside the boundary layer of the leaf (h_s), leaf temperature (T , $^{\circ}\text{C}$) and PPFD incident on the leaf (I , $\mu\text{mol m}^{-2} \text{s}^{-1}$). At 2-hour intervals the leaf water potential (Ψ_{leaf} , MPa) was also measured beginning predawn for the comparison of the water condition of the intact and excised leaves.

A meteorological station, established in an open site 30 m away from the tower, recorded precipitation ($\text{mm } 30 \text{ min}^{-1}$), PPFD, air temperature and relative humidity.

Model calculation and data analysis for feasibility examination

Using gas exchange data under ambient environmental conditions, we linearly regressed g_s with an ecophysiological parameter, $A \cdot h_s \cdot C_s^{-1}$ (Ball *et al.*, 19087), and obtained the slope, m and intercept, b ($\text{mol m}^{-2} \text{s}^{-1}$). We analysed the relationship using two time-point gas exchange data of intact leaves (under high and low light intensities, $n = 2$ light intensities $\times 18$ leaves), those after excision ($n = 36$) and diurnal data of *in situ* measurements ($n = 295$).

We constructed an ecophysiological-meteorological model to reproduce the diurnal patterns of gas exchange of each intact leaf within a cuvette (Leuning *et al.*, 1995). The model was composed of the three sub-models: (1) an ecophysiological sub-model of RuBP carboxylation and regeneration (Farquhar *et al.*, 1980; Johnson and Thornley, 1984; Harley *et al.*, 1992; Leuning *et al.*, 1995); (2) a stomatal control sub-model that determines gas exchange and sub-stomatal gas concentration (Ball *et al.*, 1987); and (3) a leaf surface energy balance sub-model the isothermal form of the Penman-Monteith equation (Leuning *et al.*, 1995; Campbell and Norman, 1998). Because we intended to model the diurnal pattern of leaf gas exchange within the cuvette, boundary layer conductance was set at the same value as the cuvette, $2.84 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$. Leaf albedo was set at 0.16, the value used for broad-leaved species in the leaf temperature calculation by the program of Li-6400. Downward longwave radiation was calculated as $\sigma(T-20)^4$ following that for clear days in Jones (1992), where σ is the Stefan-Boltzmann constant and T is air temperature (K). Even if downward longwave radiation was estimated as σT^4 instead of $\sigma(T-20)^4$, modelled leaf gas exchange rates were not significantly changed. We obtained two types of modelled gas exchange rates for each leaf: modelled rates based on intact leaf data, which used ecophysiological data before

excision as ecophysiological inputs, and those based on excised leaf data.

In the ecophysiological-meteorological model, environmental inputs are those within the cuvette of *in situ* gas exchange measurements (incident PPFD, atmospheric CO_2 concentration and air temperature, etc). Leaf ecophysiological inputs are $V_{\text{cmax}_{25}}$, $J_{\text{max}_{25}}$, R_d , θ , α , m and b of each leaf before and after excision.

To examine of the feasibility of excised leaf data as model inputs, ecophysiological inputs and modelled gas exchange rates observed from excised leaves were compared with those of intact leaves.

Results and Discussion

Climate and ecophysiology

On the day of diurnal *in situ* measurements (23 July 2009), the sky was clear and air temperature reached 33.5°C , a value similar to the July average daily maximum air temperature of this study site. The atmospheric vapor pressure deficit (VPD, kPa) also reached high levels compared to other months.

Pre-dawn leaf water potential (Ψ_{leaf} , MPa), which approximated soil water potential, was -0.26 MPa , indicating wet soil water conditions during the measurement period (Figure 1). Precipitation occurred at 2- to 3-day intervals, keeping the soil wet on each measurement day. In the daytime, Ψ_{leaf} of the intact leaves decreased and reached the daily minimum (-1.53 MPa) at 12:00–14:00. This value was lower than that from excised leaves (ca. -0.2 MPa , data not shown), indicating that the water condition of excised and intact leaves differed.

Despite the significant difference in g_s (Table I) and Ψ_{leaf} , we did not find significant differences in the ecophysiological parameters $V_{\text{cmax}_{25}}$, $J_{\text{max}_{25}}$, R_d , θ and α between intact and excised leaves (Table I). These results suggest that measurements using excised leaves would be a useful method for the precise estimation of instantaneous gas exchange traits of intact leaves (Hanba *et al.*, 1999; Medlyn *et al.*, 2002; Warren *et al.*, 2003; Cai *et al.*, 2009) under relatively high VPD for a mature

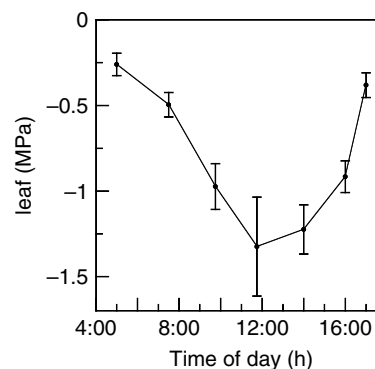


Figure 1. Diurnal patterns of leaf water potential (Ψ_{leaf} , MPa) beginning with pre-dawn period

Table I. Leaf ecophysiological parameters for gas exchange of intact leaves and those after excision (repeated measure *t*-test)

	unit		<i>n</i>	mean ± S.D.	<i>t</i> -value	<i>p</i>
$V_{\text{cmax}_{25}}$	$(\mu\text{mol m}^{-2}\text{s}^{-1})$	Intact	18	59.3 ± 7.4	1.78	0.094
		Excised	18	56.3 ± 6.9		
$J_{\text{max}_{25}}$	$(\mu\text{mol m}^{-2}\text{s}^{-1})$	Intact	18	113.1 ± 14.1	1.36	0.191
		Excised	18	107.0 ± 11.4		
A	$(\mu\text{mol m}^{-2}\text{s}^{-1})$	Intact	18	14.1 ± 1.9	−1.8	0.09
		Excised	18	14.9 ± 1.8		
g_s	$(\text{mol m}^{-2}\text{s}^{-1})$	Intact	18	0.18 ± 0.06	5.19	<0.001
		Excised	18	0.28 ± 0.06		
α	—	Intact	18	0.252 ± 0.041	0.853	0.405
		Excised	18	0.248 ± 0.033		
θ	—	Intact	18	0.81 ± 0.09	1.733	0.101
		Excised	18	0.78 ± 0.08		
R_d	$(\mu\text{mol m}^{-2}\text{s}^{-1})$	Intact	18	1.59 ± 0.22	−1.61	0.125
		Excised	18	1.80 ± 0.20		

L. edulis tree. In some species, however, a decrease in gas exchange rates and stomatal closure were observed soon after excision. Therefore, pre-screening of the focal species is required to determine whether the obtained excised leaf data are reliable (Santiago and Mulkey, 2003).

The values of g_s showed large diurnal variation from $0.01 \text{ mol m}^{-2} \text{ s}^{-1}$ before dawn to $0.35 \text{ mol m}^{-2} \text{ s}^{-1}$ at midday, and showed a strong linear relationship with $A \cdot h_s \cdot C_s^{-1}$ (Figure 2). Despite the differences in Ψ_{leaf} and g_s under light saturation between datasets (Table I), two time-point measurement data of both intact leaves and excised leaves had a linear g_s - $A \cdot h_s \cdot C_s^{-1}$ relationship similar to that of *in situ* diurnal measurements (ANCOVA, data type: $F_{2,361} = 2.59$, $P = 0.076$, data type \times covariate: $F_{2,361} = 1.60$, $P = 0.203$). This result indicates that the data of the two time-point measurement with excised leaves would be suitable for the prediction of stomatal control of intact leaves. The values of m and b were within the range of previous studies using intact leaves (Harley and Baldocchi, 1995; Sellers *et al.*, 1997; Baldocchi and Meyers, 1998; Lai *et al.*, 2000; Kumagai *et al.*, 2006).

Model simulation and *in situ* measurement of diurnal gas exchange patterns

Modelled leaf gas exchange rates based on excised leaf data were a good fit to the *in situ* measurements (Figure 3). Modelled values using excised leaf data had a 1:1 relationship with the diurnal data of *in situ* measurements for A , E , and g_s as well as the modelled values based on intact leaf data (Figure 4). Results suggested that excised leaf data should be recognized as a useful alternative estimate of intact leaf data for model simulation of gas exchange. Slight differences in ecophysiological inputs between excised leaf data and intact leaf data (Table I) did not alter the modelled

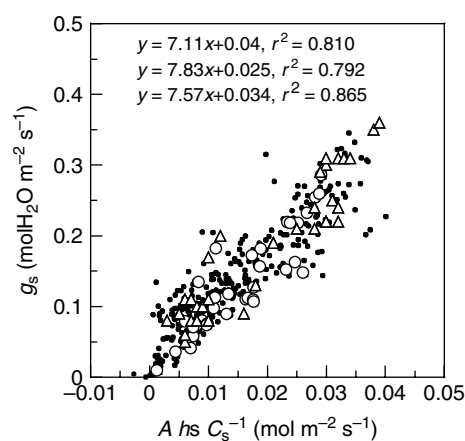


Figure 2. Correlation between stomatal conductance (g_s) and an ecophysiological parameter ($A \cdot h_s \cdot C_s^{-1}$) of the Ball-Berry model (Ball *et al.*, 1987) for data of *in situ* diurnal measurements (closed circles), two time-point measurements with excised leaf data (open triangles), and those with intact leaf data (open circles). Equations and values indicate the regression line and r^2 for each data, respectively

values. Among the input leaf traits, substitution of different m values (e.g. $m = 9$ for temperate forest trees, following Sellers *et al.*, 1997) into the model resulted in an overestimation of g_s and E , compared with the *in situ* measurement data, by 20–25% (data not shown), suggesting the importance of precise measurements of m . Modelled leaf gas exchange rates were also sensitive to changes in V_{cmax} but less so to other ecophysiological parameters (Figure 5).

This study invoked the importance of the new protocol: comparison of the modelled gas exchange rates using excised leaf data with the measured ones, rather than the comparison of each ecophysiological parameter (Santiago and Mulkey, 2003). Because model outputs are very sensitive to V_{cmax} and m , even non-significant differences in these parameters between excised leaf data and intact leaf data may confound efforts to model g_s with excised

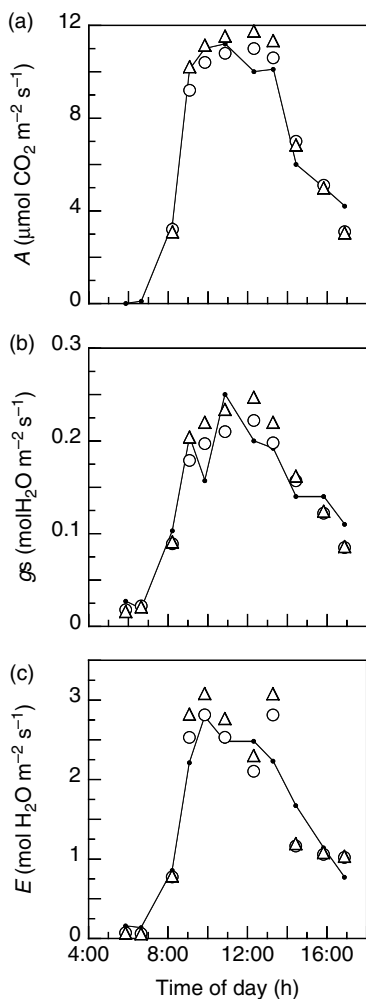


Figure 3. Diurnal pattern of (a) photosynthetic rate (A), (b) stomatal conductance (g_s) and (c) transpiration rate (E) of a leaf for *in situ* diurnal measurements (closed circles), modelled values based on two time-point measurements with excised leaf data (open triangles), and those based on intact leaf data (open circles)

leaf data. Especially in species with higher V_{cmax} , model outputs are highly sensitive to changes in m and slight differences in m can result in large error.

It should be noted that model simulation using excised leaf data is applicable only under moist soil conditions where leaf water potential is maintained at high levels. Although our study was carried out under leaf water potential greater than -2 MPa and did not detect changes in m after leaf excision, Sala and Tenhunen (1996) showed that m linearly declined with decreases in predawn leaf water potential, especially below -3 MPa. Under drought conditions, leaf excision and associated drastic improvement of leaf water potential would alter m values and model outputs from those of intact leaves.

Low r^2 in modelled g_s versus measured g_s (Figure 4) was mainly due to the variation in g_s of *in situ* measurements along the $g_s-A \cdot h_s \cdot C_s^{-1}$ regression line, rather than the imprecision of the m or b values of the excised leaf

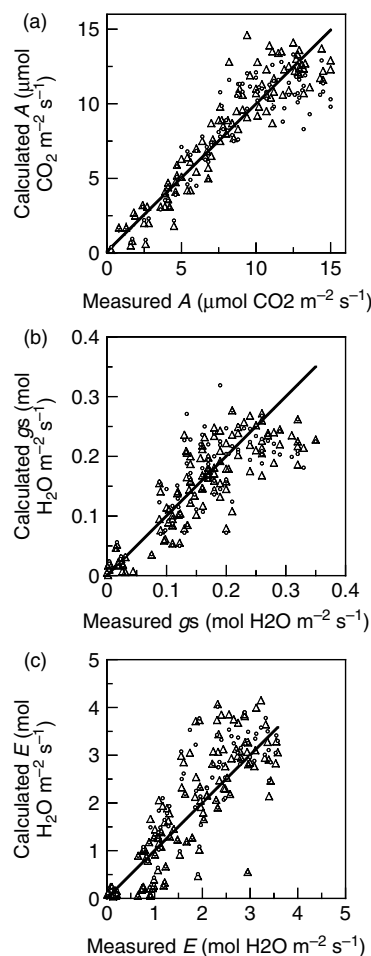


Figure 4. Correlation between the modelled gas exchange rates using an ecophysiological-meteorological model and diurnal data of *in situ* measurements for (a) photosynthetic rate (A) ($r^2 = 0.92$ and 0.88 for modelled values based on intact leaf data and those on excised leaf data, respectively), (b) stomatal conductance (g_s) ($r^2 = 0.63$ and 0.65) and (c) transpiration rate (E) ($r^2 = 0.73$ and 0.77) of the leaves. Each line represents a 1 : 1 relationship between the modelled and measured gas exchange rates. Symbols as for Figure 3

data. Even when m , computed from the $g_s-A \cdot h_s \cdot C_s^{-1}$ regression line of *in situ* measurement data, was used as an input, r^2 was not improved ($r^2 = 0.67$). By inputting m and b of each datum from *in situ* measurements as model inputs (Figure 2), r^2 of g_s was improved ($r^2 = 0.792$).

Implications for research with other environment measurement techniques

This study is valuable in that we provide a detailed protocol for prescreening the effects of excision on model simulation and a suit of measurements (Table I, Figures 3 and 4), updating the protocol proposed by Santiago and Mulkey (2003). Our knowledge is limited to only one species under wet soil conditions in a temperate forest because this is the first study to examine the feasibility of excised leaf data as a leaf gas exchange model input. Future studies that test the use of pre-screened

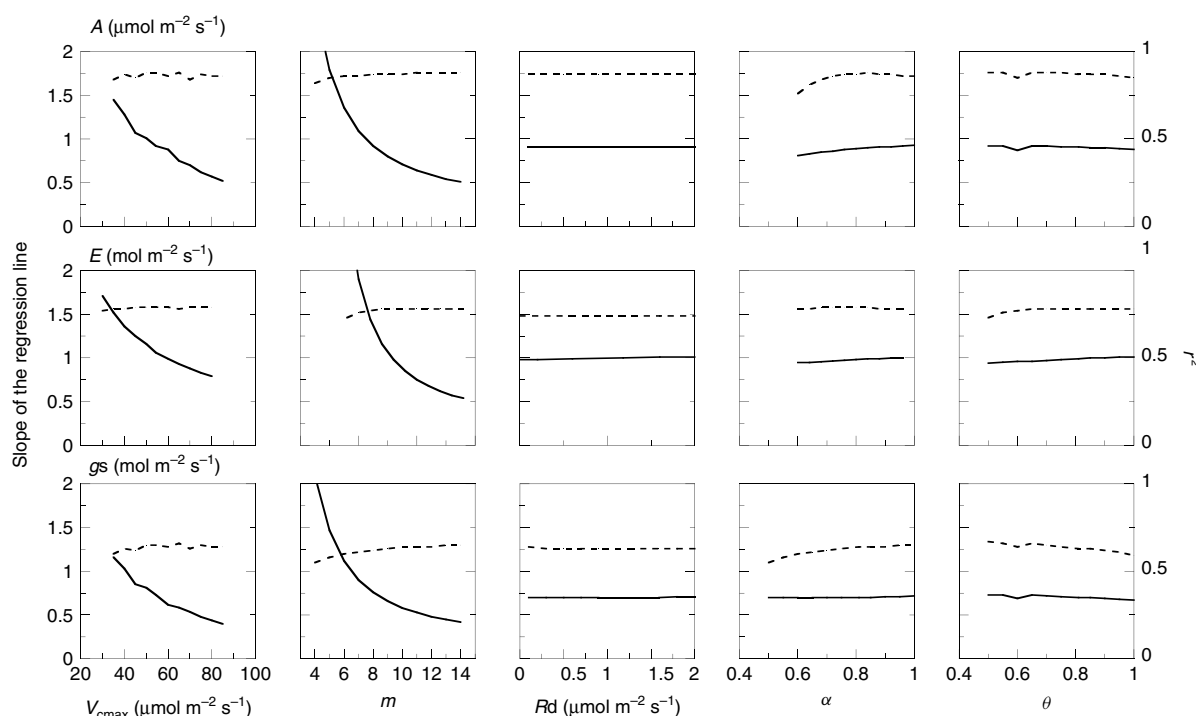


Figure 5. Sensitivity test of model outputs in response to changes in ecophysiological input of the model: V_{cmax} , m , R_d , α and θ . The slope and r^2 of the regression lines between the modelled gas exchange rates and the *in situ* measured ones are represented by the solid and broken lines, respectively

excised leaf data obtained with different methods, such as leaf sampling using a shotgun, can help to accumulate a database of methods and species that can maintain gas exchange rates and stomatal control after excision. This species list would allow researchers to monitor leaf gas exchange without towers by frequently excising canopy leaves, in combination with other environmental measurements, such as sap flow measurements (Kim *et al.*, 2008).

The feasibility of model prediction with excised leaves should encourage researchers who want to estimate gas exchange at study sites without canopy-accessing towers. To collect intact leaf data at study sites composed of a mosaic of different stands (e.g. plantations composed of various stand ages and tree densities) for the prediction of gas exchange at large spatial scales (Mackay *et al.*, 2010), we would need to build a tower in each stand. Using excised leaves, ecophysiological data could be collected at larger spatial scales without building many towers.

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References

- Baldocchi D, Meyers T. 1998. On using eco-physiological, micrometeorological and biogeochemical theory to evaluate carbon dioxide, water vapor and trace gas fluxes over vegetation: a perspective. *Agricultural and Forest Meteorology* **90**: 1–25.
- Ball JT, Woodrow LE, Berry JA. 1987. A model predicting stomatal conductance and its contribution to the control of photosynthesis under different environmental conditions. In *Progress in Photosynthesis Research*, Biggens L (eds). Martinus Nijhoff Publishers: Dordrecht; 221–224.
- Cai ZQ, Schnitzer SA, Bongers F. 2009. Seasonal differences in leaf-level physiology give lianas a competitive advantage over trees in a tropical seasonal forest. *Oecologia* **161**: 25–33.
- Campbell GS, Norman JM. 1998. *An introduction to environmental biophysics*. Springer: New York.
- Domingues TF, Meir P, Feldpausch TR, Saiz G, Veenendaal EM, Schrodte F, Bird M, Djagbletey G, Hien F, Compaore H, Diallo A, Grace J, Lloyd J. 2010. Co-limitation of photosynthetic capacity by nitrogen and phosphorus in West Africa woodlands. *Plant Cell and Environment* **33**: 959–980.
- Ewers BE, Oren R, Kim HS, Bohrer G, Lai CT. 2007. Effects of hydraulic architecture and spatial variation in light on mean stomatal conductance of tree branches and crowns. *Plant Cell and Environment* **30**: 483–496.
- Farquhar GD, Caemmerer SV, Berry JA. 1980. A biochemical-model of photosynthetic CO_2 assimilation in leaves of C-3 species. *Planta* **149**: 78–90.
- Ford CR, Hubbard RM, Kloeppel BD, Vose JM. 2007. A comparison of sap flux-based evapotranspiration estimates with catchment-scale water balance. *Agricultural and Forest Meteorology* **145**: 176–185.

- Hanba YT, Miyazawa SI, Terashima I. 1999. The influence of leaf thickness on the CO₂ transfer conductance and leaf stable carbon isotope ratio for some evergreen tree species in Japanese warm-temperate forests. *Functional Ecology* **13**: 632–639.
- Harley PC, Baldocchi DD. 1995. Scaling carbon-dioxide and water-vapor exchange from leaf to canopy in a deciduous forest. 1. Leaf model parametrization. *Plant Cell and Environment* **18**: 1146–1156.
- Harley PC, Thomas RB, Reynolds JF, Strain BR. 1992. Modeling photosynthesis of cotton grown in elevated CO₂. *Plant Cell and Environment* **15**: 271–282.
- Irvine J, Perks MP, Magnani F, Grace J. 1998. The response of *Pinus sylvestris* to drought: stomatal control of transpiration and hydraulic conductance. *Tree Physiology* **18**: 393–402.
- Joesting HM, McCarthy BC, Brown KJ. 2009. Determining the shade tolerance of American chestnut using morphological and physiological leaf parameters. *Forest Ecology and Management* **257**: 280–286.
- Johnson IR, Thornley JHM. 1984. A model of instantaneous and daily canopy photosynthesis. *Journal of Theoretical Biology* **107**: 531–545.
- Jones HG. 1992. *Plants and Microclimate*. Cambridge University Press: Cambridge.
- Kim HS, Oren R, Hinckley TM. 2008. Actual and potential transpiration and carbon assimilation in an irrigated poplar plantation. *Tree Physiology* **28**: 559–577.
- Kumagai T, Ichie T, Yoshimura M, Yamashita M, Kenzo T, Saitoh TM, Ohashi M, Suzuki M, Koike T, Komatsu H. 2006. Modeling CO₂ exchange over a Bornean tropical rain forest using measured vertical and horizontal variations in leaf-level physiological parameters and leaf area densities. *Journal of Geophysical Research-Atmospheres* **111**: D10107.
- Kume A, Satomura T, Tsuboi N, Chiwa M, Hanba YT, Nakane K, Horikoshi T, Sakugawa H. 2003. Effects of understory vegetation on the ecophysiological characteristics of an overstory pine, *Pinus densiflora*. *Forest Ecology and Management* **176**: 195–203.
- Lai CT, Katul G, Oren R, Ellsworth D, Schafer K. 2000. Modeling CO₂ and water vapor turbulent flux distributions within a forest canopy. *Journal of Geophysical Research-Atmospheres* **105**: 26333–26351.
- Leuning R. 1995. A critical-appraisal of a combined stomatal-photosynthesis model for C-3 plants. *Plant Cell and Environment* **18**: 339–355.
- Leuning R, Kelliher FM, Depury DGG., Schulze ED. 1995. Leaf nitrogen, photosynthesis, conductance and transpiration—scaling from leaves to canopies. *Plant Cell and Environment* **18**: 1183–1200.
- Lloyd J, Grace J, Miranda AC, Meir P, Wong SC, Miranda BS, Wright IR, Gash JHC, McIntyre J. 1995. A Simple calibrated model of Amazon rain-forest productivity based on leaf biochemical-properties. *Plant Cell and Environment* **18**: 1129–1145.
- MacKay DS, Ahl DE, Ewers BE, Gower ST, Burrows SN, Samanta S, Davis KJ. 2002. Effects of aggregated classifications of forest composition on estimates of evapotranspiration in a northern Wisconsin forest. *Global Change Biology* **8**: 1253–1265.
- Mackay DS, Ewers BE, Loranty MM, Kruger EL. 2010. On the representativeness of plot size and location for scaling transpiration from trees to a stand. *Journal of Geophysical Research-Biogeosciences* **115**: G02016.
- Medlyn BE, Loustau D, Delzon S. 2002. Temperature response of parameters of a biochemically based model of photosynthesis. I. Seasonal changes in mature maritime pine (*Pinus pinaster* Ait.). *Plant Cell and Environment* **25**: 1155–1165.
- Miyazawa Y, Otsuki K. 2010. Comparison of sapling-level daily light capture and carbon gain between a temperate deciduous and a co-occurring evergreen tree species in the growing season and in winter. *Functional Plant Biology* **37**: 215–222.
- Reich PB, Ellsworth DS, Uhl C. 1995. Leaf carbon and nutrient assimilation and conservation in species of differing successional status in an oligotrophic Amazonian forest. *Functional Ecology* **9**: 65–76.
- Sala A, Tenhunen JD. 1996. Simulations of canopy net photosynthesis and transpiration in *Quercus ilex* L under the influence of seasonal drought. *Agricultural and Forest Meteorology* **78**: 203–222.
- Santiago LS, Mulkey SS. 2003. A test of gas exchange measurements on excised canopy branches of ten tropical tree species. *Photosynthetica* **41**: 343–347.
- Sellers PJ, Dickinson RE, Randall DA, Betts AK, Hall FG, Berry JA, Collatz GJ, Denning AS, Mooney HA, Nobre CA, Sato N, Field CB, HendersonSellers A. 1997. Modeling the exchanges of energy, water, and carbon between continents and the atmosphere. *Science* **275**: 502–509.
- von Caemmerer S, Evans JR, Hudson GS, Andrews TJ. 1994. The kinetics of ribulose-1,5-bisphosphate carboxylase/oxygenase in-vivo inferred from measurements of photosynthesis in leaves of transgenic Tobacco. *Planta* **195**: 88–97.
- Wang YP, Leuning R. 1998. A two-leaf model for canopy conductance, photosynthesis and partitioning of available energy I: Model description and comparison with a multi-layered model. *Agricultural and Forest Meteorology* **91**: 89–111.
- Warren CR, Ethier GJ, Livingston NJ, Grant NJ, Turpin DH, Harrison DL, Black TA. 2003. Transfer conductance in second growth Douglas-fir (*Pseudotsuga menziesii* (Mirb.)Franco) canopies. *Plant Cell and Environment* **26**: 1215–1227.