Physiological control of carbon and water fluxes in the Chequamegon National Forest, its variability and consequences

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Abstract

Stomatal function and photosynthetic capacity in the Chequamegon National Forest display much variability within and between individuals, species, seasons or years, and sites (upland forest, lowland forest, and wetland). Significant partitioning of the variability is between overstory and understory, by species, and by drought (1997). The remaining large variability on any site must be incorporated into flux models for the net carbon storage and site hydrology. Simulations with our observed ranges of Ball-Berry parameters (found to best fit stomatal behavior) and photosynthetic capacity (as $V_{c,\text{max}}$ referred to 25°C) were undertaken in order to estimate (1) which of these parameters (their statistical distributions) are most important to measure accurately and (2) whether integrated fluxes of CO$_2$ and water respond linearly to parameter values and thus can be modelled with single, averaged values of parameters. We find a high flux control coefficient (0.35) for total stomatal conductance $g_s$ over water flux, and a weaker one near 0.10 for carbon flux, for representative summertime conditions. Stomatal response to humidity and to photosynthetic rate appears to be important, in contrast to simpler stomatal models, but the quantitative importance is seen only in those simulations that resolve distributions of irradiances on leaves rather than single average irradiances. Carbon flux is significantly and nonlinearly sensitive to variations in Ball-Berry slope $m$ alone, but flux becomes highly insensitive when the strong negative correlation of slope with intercept $b$ is accounted. Carbon flux remains sensitive to $V_{c,\text{max}}^{25}$, but in linear fashion, and water flux appears linearly dependent upon both $m$ and $V_{c,\text{max}}^{25}$. The latter may be taken as the only two independent parameters, eliminating $b$. Thus, $V_{c,\text{max}}^{25}$ seems most important for large-scale simulations with more detailed models such as SiB2. Indirect control of carbon flux by water fluxes that can lead to water stress may be as important as direct control of carbon flux, in certain years. In detailed simulations, single average values of parameters should suffice, given the linearity of fluxes. Additional field measurements indicate that photosynthetic downregulation in response to high irradiance or high leaf temperature should not be significant under almost all summertime conditions.
Introduction

The Chequamegon National Forest is of great interest as a paradigm of the proposed North American CO$_2$ sink that has been inferred from inversions of global CO$_2$ mixing ratios and isotopic composition (Fan et al. 1998; see reanalysis by Field and Fung 1999). The site is of independent interest for its processes of ecosystem regeneration (e.g., Buckley et al. 1998) and hydrology. The carbon sink activity may derive heavily from the forest being immature and aggrading, as is a large area of North American forest (Houghton et al. 1999, 2001; Caspersen et al. 2000). The sink activity may also depend upon physiological control of CO$_2$ and water fluxes by stomata and by photosynthetic-capacity regulation.

Our focus is on this physiological control. We have made extensive surveys of leaf gas exchange on leaves in situ, including those at the tops of dominant trees that we access with boom lifts (Fig. 1). Using several protocols, we measure stomatal conductance and CO$_2$ assimilation under a wide variety of conditions of leaf irradiance, humidity, and temperature, primarily. These data have revealed large physiological variations, even at one site in one season. Such variability is not included in detailed flux models such as SiB2 (Sellers et al. 1996a,b; see article by Denning et al. in this issue). One may ask if it suffices to use averaged values of stomatal and photosynthetic parameters, which is equivalent to asking if fluxes are linear in the parameters. Concurrently, one may ask if the average values employed in detailed flux models reflect the distributions of parameter values that we have observed. The consequences of errors in mean and variance in parameters can be amplified, in estimating the carbon sink from on-site flux measurements as the slight imbalance between gross assimilation and ecosystem respiration. Similarly, errors in representing physiological control may notably affect the estimation of regional fluxes from atmospheric mixing ratios, particularly if physiological control amplifies the "rectifier effect" (Denning et al. 1995, 1999). We use simplified models that capture essential features of physiology interacting with environment to estimate physiological flux control and the effects of averaging and of parameter offsets.

Physiological control may also be exerted via diurnal variation in the downregulation of photosynthetic electron transport ("photoinhibition") by excess light interception and the
downregulation of carboxylation capacity by high temperature. We have made limited surveys of these effects, to indicate that they are of minor quantitative significance.
Access to leaves for measuring gas exchange, temperature, irradiance: Understory vegetation was readily accessed. Dominant trees up to 22 m were accessed with a self-powered boom lift that possesses a telescoping arm (boom) capable of extending 26 m at an angle up to 60° from horizontal. The boom lift achieves stability by great mass (3600 kg) over a modest stance (about 3 x 2 m). The great mass necessitates that the terrain be near level (< 10° slope) and firm. Thus, operations are restricted to the vicinity of good roads in the absence of very wet soil or heavy litter layers.

Leaf gas exchange: Basic closed-mode measurements: a standard LI-6200 portable photosynthesis system (LI-COR, Inc., Lincoln, NE, USA) was used in accordance with the manufacturer’s recommendations. Particular care was taken to assure that leaf water flux was measured primarily by accurately measurable airflow through desiccant (a high magnitude of "K"). Leaves were measured intact in normal orientations after long acclimation to ambient environment. On occasion, the environment was changed artificially by shading or lighting, again allowing lengthy acclimation times. Multiple 10-s observations of fluxes were made and either averaged when similar or chosen for low variance in all measured variables otherwise.

Open-mode gas exchange: The LI-6200 (was replumbed so that incoming air passed through a source of controlled humidity (LI-610 humidity generator, in which air bubbles though T-controlled water), then into the LI-6200 IRGA reference chamber, then into the cuvette with the leaf, then back to the IRGA sample chamber before exiting to free air. Before entering the IRGA sample chamber, all or part of the air exiting the cuvette passes through a dew-point hygrometer (model Hygro M-1 with model 1111H sensor, General Eastern, Woburn, MA, USA). We corrected for expansion of air that causes a drop in water-vapor pressure between the dew-point generator and the cuvette, using a digital manometer whose output is sent to the LI-6200 console, along with the humidity-generator temperature and the hygrometer output. Using a new monitoring program that we wrote for the LI-6200 console, this complete set of information allows the LI-6200 console to compute assimilation \( A \), in situ transpiration \( E \) (not used to estimate free-air transpiration), stomatal conductance (\( g_s \)), water vapor pressure and relative humidity (\( h_s \))
at the leaf surface beneath the boundary layer, and CO$_2$ mixing ratio at the leaf surface (C$_s$) in real time. This novel system is tested for stability and for air leaks by checking that computed water-vapor partial pressure (e$_a$) and CO$_2$ mixing ratio entering the cuvette closely equal the corresponding values at the air exit when no leaf is present. Acceptable offsets in ea and Ca are equivalent to conductances of 0.01 mol m$^{-2}$s$^{-1}$ and assimilations of 0.3 mol m$^{-2}$s$^{-1}$. A complete manual for operating a LI-6200 system in this mode has been prepared and is available from the authors. At times, artificial light was required to maintain gas exchange conditions. We used an array of 3 quartz-halogen lamps (12 VDC, 50 W each), filtering out excess thermal radiation with a Plexiglas filter.

*Dark respiration by leaves:* this was estimated by fully shielding the cuvette with aluminum foil for 1 min or more and performing either closed-mode or open-mode gas exchange.

*Leaf areas:* these were measured both geometrically, using dimensions apparent with the leaf in the cuvette, and by direct measurement on the section of leaf marked as inside the cuvette (allowing for the cuvette gasket), using an LI-2000 leaf area meter.

All equipment was modified to operate in the field on 12VDC supplied by marine batteries (which allow deep cycling of their charge status without damage).

*Estimation of photosynthetic capacity, V$_{c,max}$, from gas-exchange data:* The theory is best illustrated in the limit of high leaf irradiance, at which condition the assimilation rate is simply expressed as

$$A = V_{c,max} \frac{(C_i - \Gamma)}{(C_i + K_{CO})} \Rightarrow V_{c,max} = A \frac{(C_i + K_{CO})}{(C_i - \Gamma)}. \quad (1)$$

Here, $C_i$ is the CO$_2$ partial pressure in the leaf interior (after passing the stomatal resistance), $\Gamma$ is the CO$_2$ partial pressure at compensation, and $K_{CO}$ is the effective Michaelis constant for CO$_2$ binding in the presence of ambient levels of oxygen. Both $\Gamma$ and $K_{CO}$ are otherwise functions only of leaf temperature and are computed using the formulas of de Pury and Farquhar (1997). Commonly, $V_{c,max}$ is fitted from a series of measurements of $A(C_i)$, though we use a single measurement that only moderately reduces accuracy (Niinemets *et al.* 1999). At finite irradiances $I_L$, assimilation is expressible in terms of a combination of the light-saturated rate above and the light-limited rate, $A_{LL}$, as
\[ \theta A^2 - A (A_{\text{sat}} + A_{\text{LL}}) + A_{\text{sat}}A_{\text{LL}} = 0. \]  

(2)

The convexity parameter \( \theta \) is commonly about 0.8, as we estimated from several light-response curves. The light-limited rate is expressible as \( Q_0I_L \), with the initial yield \( Q_0 \) as \( Q_{00}(C_i - \Gamma)/(C_i + 2\Gamma) \), taking \( Q_{00} \) as about 0.0732 for leaves of normal pigmentation (Ehleringer and Björkman 1977). With this form for \( A \), we obtain a quadratic equation for \( V_{\text{c,max}} \) when provided with measurements of \( A, C_i, T_L, \) and \( \theta \). This fit is only usefully accurate when \( A_{\text{LL}} \) is at least as large as the light-saturated rate. The value of \( V_{\text{c,max}} \) can be scaled to a uniform reference temperature of 25°C, using the temperature activation factor of de Pury and Farquhar (1997).

**Translation of \( A \) and surface variables to free-air conditions:** In fitting stomatal conductance to the Ball-Berry (Ball et al. 1987) or allied models, one uses leaf temperature, \( C_i \), and ambient water-vapor pressure \( e_a \) and \( \text{CO}_2 \) partial pressure \( C_a \). These variables are all changed by placing the leaf in the cuvette, where energy balance components as well as \( e_a \) and \( C_a \) may differ from those to which the leaf had acclimated. If \( g_s \) acclimates rapidly to the new cuvette conditions, we may compute the Ball-Berry or related indices using the values measured in the cuvette. In the other limit, \( g_s \) does not change and it must be related to original, "free-air" conditions. Required data include original values of leaf temperature, \( e_a \), and \( C_a \), as well as original leaf boundary-layer conductance, \( g_{\text{b,air}} \) estimated commonly from windspeed and leaf dimension (Nobel 1991). One may equate the transport equation for assimilation \( A \) with the carboxylation equation for \( A \). At light saturation, this takes the form

\[ A = g_{\text{tot,CO}_2}(C_a - C_i)/P = V_{\text{c,max}} \frac{(C_i - \Gamma)}{(C_i + K_{\text{CO}_2})}. \]  

(3)

Here, total conductance for \( \text{CO}_2 \) from ambient air to interior, \( g_{\text{tot,CO}_2} \), is composed as \( 1/(1.6/g_s + 1.37/g_{\text{b,air}}) \) (Ball 1987); \( P \) is the total air pressure. This is solved as an equation for \( C_i \), which is back-substituted to estimate \( A_0 \). When one uses the full Eq. (2) for assimilation, a quartic equation for \( C_i \) results; it is readily solved by binary search.

**Auxiliary environmental variables:** We measured leaf water potential, psiL, on many of the same leaves used for gas exchange, using a standard pressure bomb (SoilMoisture Corp., Santa Barbara, CA). The data showed little correlation with \( g_s \) and are not used in the present analysis. Spatial distributions of leaf irradiance and long-time averages of irradiance on single leaves were
measured using leaf-mounted photodiodes (updated version of system developed by Gutschick et al., 1985). Total transpiration by leaf assemblages on branches and whole trees was measured with sapflow gauges employing both commercial equipment and equipment that we designed and built. These data are used in scaling up measurements to branch and tree and will be treated in later publications. Canopy structural data on leaf area index, leaf clumping (see, e.g., Chen 1996, Kucharik et al. 1998; Gutschick 1991), and leaf angle distributions were obtained with a leaf canopy analyzer (LAI-2000, LI-COR, Inc., Lincoln, NE, USA) and by image analysis on upward-looking photographs (our own program).

**Simulation model for stand fluxes of CO₂ and water vapor:** This model is detailed sufficiently in the text, in order to highlight its features.
Results and Discussion

We used several methods to measure the patterns of stomatal control in diverse species and locations. Leaves respond to changes in diverse factors: PAR irradiance (PPFD), humidity, ambient CO$_2$, temperature, and boundary-layer conductance (related to windspeed). It is impractical to explore ranges in all these environmental conditions. Thus, we draw on past research to fit stomatal conductance to simple, empirical indices that incorporate all these factors. Two such empirical fits are (1) the model of Ball et al. (1987), hereafter called "Ball-Berry" and indicated by subscript "BB":

$$g_s \approx m \frac{A h_s}{C_s} + b$$

(4)

and (2) the model of Leuning (1997)

$$g_s \approx m \frac{A}{C_s(1 + D/D_0)} + b$$

(5)

In these formulas, $m$ and $b$ are empirical slopes and intercepts, respectively against the indices $I_{BB} = A h_s/C_s$ or $I_L = A/[C_s'(1 + D/D_0)]$. Here, $A$ is the CO$_2$ assimilation rate, commonly called photosynthetic rate and measured in $\mu$mol m$^{-2}$s$^{-1}$; $h_s$ is the relative humidity at the leaf surface, beneath its boundary layer; it is the partial pressure of water vapor there, $e_s$, divided by the saturated vapor pressure at leaf temperature, $e_{sat}$. The quantity $C_s$ is the mixing ratio or mole fraction of CO$_2$ at the leaf surface, commonly expressed in $\mu$mol mol$^{-1}$ (parts per million). The primed version, $C_s'$, is $C_s - \Gamma$, where $\Gamma$ is the CO$_2$ compensation pressure (see, e.g., Farquhar et al. 1980). The quantity $D$ is the vapor-pressure deficit, $e_s - e_a$. Finally, $D_0$ is a third empirical constant in the Leuning model.

Many different protocols can be used to change the operating point of a leaf - changing leaf temperature ($T_L$), or humidity, or PPFD, etc. Many experiments (ibid.) have shown that responses fall close to a common line when $g_s$ is plotted against the indices $I_{BB}$ or $I_L$. Thus, we typically varied only one major variable to obtain $g_s$ as a function of index I: (1) $T_L$ was varied on leaves allowed to reach steady state at constant humidity $e_a$ and PPFD; (2) humidity $e_a$ was varied while $T_L$ and PPFD were held constant; (3) PPFD was varied artificially. These three
methods require an open-mode gas-exchange system and long times of observation and acclimation. A fourth method was to find a leaf or leaves in varied PPFD (and perhaps T_L, e_a) and then make rapid measurements in closed-mode gas exchange. All four methods appear to give similar results on given leaves.

Photosynthetic capacity is perhaps best expressed as maximal carboxylation rate, V_{c,max}. It is readily estimated from measurements of A simultaneously with leaf-internal CO_2 partial pressure, C_i, and of T_L, using the formulas of Farquhar et al. (1980; updated by dePury and Farquhar 1997; see "Materials and Methods"). Both C_i and T_L are obtained during gas-exchange measurements. We use standard temperature scaling (dePury and Farquhar 1997) to refer V_{c,max} to a common reference temperature of 25°C, as V_{c,max}^{25}. We take V_{c,max}^{25} as a measure of investment in Rubisco enzyme. Corrections for partial light-saturation of assimilation are described in Materials and Methods. We use a single measurement of A, C_i, and T_L to invert the carboxylation equations to V_{c,max} rather than a series of measurements of A(C_i); the accuracy relative to the latter method is not seriously reduced (Niinemets and Tenhunen 1997). In some measurement series, we exposed leaves to sufficiently high T_L to induce thermal deactivation of carboxylation capacity. This tested the possibility that such deactivation limited A at times of the day and seasons. We expressed V_{c,max} then as

\[ V_{c,max} = f(T) V_{c,max}^{25} \exp(64000 * (T_L - 25)/(R * T * 298.2)). \]

Commonly, f(T) is nearly flat up to a critical T, at which point it declines approximately linearly with T (Fig. 2). We note that the critical temperatures exceed all T_L that we measured radiatively while doing gas-exchange surveys. In simulations discussed below, there are rare times when T_L does reach such heights. We provisionally conclude that thermal deactivation is of very small significance in this forest.

Another measure of photosynthetic acclimation is the downregulation of electron transport capacity. We measured chlorophyll fluorescence with a commercial instrument to extract the quantity F_v/F_m, which indicates the degree of downregulation (Schreiber et al. 1995). Figure 3 shows that downregulation was moderate and rapidly reversed; it was appropriate to the excess PPFD and does not indicate persistent downregulation or photodamage. Similar findings for forest trees have been reported by Niinemets et al. (1998a). The exceptions were understory plants,
which exhibited significant downregulation at all times in midday.

At the Chequamegon National Forest, we sampled stomatal and photosynthetic control in three major ecosystems: upland forest ("UL"), near Willow Springs, a mesic forest of mixed hardwoods with seasonal drying of surface soil, though rarely of deep soil; (2) lowland forest, close to the WLEF radio tower ("tall tower", instrumented for flux measurement; Bakwin et al. 1998; Yi et al. 2000); a mixed deciduous - coniferous forest with a more open canopy and an understory of diverse forbs, grasses, and tree saplings; and (3) wetlands near the WLEF tower, composed primarily of alders (Alnus rubrum) and willows (Salix species) as an overstory, with minor cover of grasses and forbs. We sampled both overstory dominants and understory species at the sites, as well as different species within each site + story combination, different individual plants of each species, and sometimes different individual leaves on individual plants.

We compared the BB and Leuning models for goodness of fit to our data. Table 1 shows that the BB method was almost always notably superior, achieving fits with $r^2$ well over 0.5 and commonly over 0.7-0.8. This marked superiority was unexpected, given results of other researchers on different plant species and locations (Leuning 1995). The best fits for the Leuning model commonly collapsed to either $D_0$ very small (thus, $g_s$ proportional to $1/D$) or very large (no response to humidity). Thus, we used the BB model in the rest of our analyses.

A clear feature of stomatal and photosynthetic control in the Chequamegon National Forest is its diversity. Table 2 presents simultaneous measures of the Ball-Berry slope $m$, the intercept $b$, and $V_{c,max}^{25}$. The number of measurements of $V_{c,max}^{25}$ is even larger, given that the BB model was often fitted with data from several leaves of varied $V_{c,max}^{25}$ that were taken to represent a common stomatal behavior. Some of the notable variability is statistically significant among groups when one examines the three parameters separately (Table 3). One feature is that $V_{c,max}^{25}$ was reduced in the upland forest in the drought year of 1997, as one might expect; while years 1998 and 1999 are not different for the two species studied in common those years (see "contrasts," at bottom of table). Trees with low leaf nitrogen content (alder, spruce) have low $V_{c,max}^{25}$. All three locations (upland forest, lowland forest, wetland) are similar in $m$, as also in $b$. The understory plants are markedly low in $m$ and high in $b$ (unresponsive stomata, with high basal value of conductance that has little significance for water loss in the still, humid environment). Species that
have high $m$ typically have high $b$, as seen in the grouping by species.

Nonetheless, a considerable amount of variability in each parameter is not explained by site, season, or species. We deem it important to estimate the functional significance of this variability. In particular, if the fluxes of CO$_2$ and of water vapor are nonlinear in $m$, $b$, and $V_{c,\text{max}}^{25}$, then it become important to sample the distributions of parameters more carefully in order to estimate their variances as well as their means; the sampling effort becomes larger.

While the three principal parameters each have wide variances, the variations are not completely independent. The correlations assume some importance, as flux modelling shows later. To explore the correlations among parameters, a compact representation is via three-dimensional plots (Fig. 4). One sees readily that the overstory and understory plants occupy distinct spaces in such a plot. Additionally, Fig. 4c shows that the parameters $m$ and $b$ alone appear to be correlated:

$$b \approx S_b m + b_0.$$ (7)

Such a correlation is "necessary." Plants with low slopes $m$ can only have conductances that are usably large for photosynthesis if their intercepts are large. The magnitude of the "super-slope" $S_b$ is about -0.028 for overstory plants in the upland forest ($r= -0.668$, $N=13$, $p=0.018$); it is about -0.0165 in lowland forest ($r= -0.836$, $N=10$, $p=0.003$). The correlation is not statistically significant in wetland overstory, but only four data points are available. The correlation also appears to have great functional significance in flux control, as will be explored later with simulations of whole-stand fluxes from overstory vegetation.

**Functional significance of control and its variation: a flux model**

Companion papers in this issue offer interpretive models of landscape fluxes (XXXX), using in particular the well-developed SiB2 model (Sellers et al. 1996a,b). The SiB2 model reproduces observed time-series of fluxes rather closely, while using generic estimates of $m$, $b$ and $V_{c,\text{max}}^{25}$ that were derived for this forest type with very limited sampling of stomatal and photosynthetic control. Is the good fit fortuitous? Are only average parameter values significant or also their variances? If the variances are important, then a single simulation with mean parameter values will
have reduced accuracy. Is one of the three parameters more important to measure accurately than the other two? More generally, how much control over fluxes is exerted by the plants, particularly their physiology \((m, b\) and \(V_{c,max}^{25}\)) as well as by their development and structure? Such questions are important in investigating why this forest and others like it are net C-sinks, as well as how interpretations of broader-scale patterns in atmospheric CO\(_2\) are modified by the "rectifier effect" in surface conductances and fluxes (Denning et al., 1995, 1999). (Here, we focus on physiological parameters, while structure is undoubtedly important. Tall trees are better coupled to the environment than is short vegetation, because their boundary layers are more conductive [Jarvis and McNaughton 1986; see also Raupach 1988 on coupling enhanced by surface sensible heat flux]. The immaturity of the forest may also be important, because carbon-store development and leaf area index have not saturated and allow a net C-sink to appear [Houghton 1999].)

Exploration of how the physiological parameters control flux is possible with a flux model of a canopy. We developed a simplified model that uses our model of physiological control and essential elements of the distribution of leaf environments. The model includes:

1. The Ball-Berry model of stomatal control;
2. A realistic model of assimilation, essentially that of de Pury and Farquhar (1997), which makes \(A\) respond to irradiance, \(C_i\), and \(T_L\). Here, \(C_i\) is determined make the transport expression for \(A\) equal to the enzymatic expression for \(A\). In the simple case of light-saturated assimilation, we have Eq. (3) in "Materials and Methods." The boundary-layer conductance is reduced by inclusion of the boundary-layer resistance for the whole canopy, \(1/g_{b,c}\), in series with the resistance of the leaf, \(1/g_{b,L}\). Both boundary conductances are functions of windspeed \(u\) measured at a reference height of 30 m: \(g_{b,L} = 0.132 \sqrt{(u/d_L)}\), with \(d_L\) = leaf characteristic linear dimension, and \(g_{b,c} = 0.2 \times u\). The numerical constant for \(g_{b,L}\) is appropriate for a leaf partly shielded in the canopy; the constant for \(g_{b,c}\) is adjusted from that reported in SiB2 (Sellers et al. 1996a) to use 30 m as a reference height.

This model requires that we specify \(T_L\), the carboxylation capacity \(V_{c,max}^{25}\), and the meteorological variables. Then, Eq. (3) becomes a quadratic equation in \(C_i\) which may be solved; \(C_i\) can be back-substituted to determine \(A\). We use a more accurate form for assimilation, Eq. (2), that describes the transition between light-limited and light-saturated rates. In this case, Eq. (3) is
generalized to a quartic equation in $C_i$. We choose the convexity parameter $\theta = 0.8$ from a series of experimental measurements in this and similar forests.

(3) Steady-state energy balance of the leaf. We account for shortwave energy absorption (absorptances of 0.85 in the PAR, 0.35 in the near infrared) and for thermal infrared absorption and emission. Sky radiation at energy flux density $Q_{\text{sky}}$ enters at an effective temperature $30^\circ C$ below air temperature. We calculate an effective depth $L_{\text{eff}}$ in the canopy at which the mean leaf is found, and thus, a fraction of sky "viewed" from the mean leaf, $f_{\text{sky}}$. The mean TIR flux density on the leaf is $f_{\text{sky}}Q_{\text{sky}} + (2 - f_{\text{sky}})Q_{\text{veg}}$, with TIR flux density from vegetation occurring at mean air temperature.

(4) A distribution of irradiances on leaves, arising from differences in depth in the canopy and in leaf orientation. At any depth, the diffuse skylight is estimated as $D_0 = D_{00}e^{-L}$, uniformly for all leaves (Gutschick 1984). The direct solar beam penetrates to depth $L$ as cumulative leaf area index with probability $\exp(-0.5L/\cos\theta_s)$, where $\theta_s$ is the solar zenith angle computed from local latitude, Julian date, and solar time. We assume a uniform distribution of leaf zenith angles and of azimuthal angles; this is consistent with our photographic surveys at the tops of upland and lowland forest. Thus, sunlit leaves at depth $L$ experience a uniform probability distribution of irradiances from $D_0$ to $D_0 + I_{00}$ (Gutschick 1984). Here, $I_{00}$ is the flux density of the direct solar beam normal to its direction of propagation. We estimate $D_0$ and $I_{00}$ from hourly records of total PAR flux density on a horizontal sensor. For better accuracy, we characterize each hour as having a fraction of clear time, in which both diffuse and direct flux are present, and a cloudy fraction with only diffuse radiation. We use initial à priori estimates of $I_{00}$ and $D_{00}$ to estimate clear fraction, and then adjust $I_{00}$ or $D_{00}$ if this fraction is negative or above unity.

From the irradiance model (4), we obtain a histogram of irradiances from zero to a maximum value of $D_{00} + I_{00}$. For each bin of the histogram, we simulate assimilation and transpiration by simultaneously solving the first three models. These are nonlinear algebraic models, so that an iterative numerical method is used: (1) we estimate the range of magnitudes in which the value of $g_s$ may lie; (2) for any chosen value of $g_s$, we can solve the energy balance equation for $T_L$; (3) we use this $T_L$ to compute the temperature-activated values of $V_{c,\text{max}}$, $\Gamma$, and $K_{co}$, the effective Michaelis constant of Rubisco for CO$_2$; (4) we may now solve Eq. (3), generalized for
convexity, for $C_i$ and thus for $A$; (5) we compute the surface relative humidity $h_s$ and the CO$_2$ partial pressure $C_s$ from simple transport equations; (6) we test if this value of $g_s$ is consistent with the BB equation, by computing $F = g_s - (mA h_s / C_s)$; we seek the value of $g_s$ that solves $F = 0$ to acceptable accuracy via a binary search in $g_s$. Special program-flow controls enable re-estimation of search limits if a solution is not found.

This model is in the spirit of a big-leaf model, generalized to represent the distribution of irradiances and the attendant variations in energy balance, assimilation, and transpiration. These variations are important; use of a single, average irradiance of leaves changes $A$ and $E$ by up to 30% and variably so with solar elevation, etc. The improved representation of TIR flux in the energy balance also changes the simulation from the common big-leaf model (in those versions in which TIR flux is resolved at all). The more reasonable TIR irradiance raises mean $T_L$, and more so at high solar elevation; values of $E$, in particular, are changed (increased) about 5% over our simulation over 16 days of weather records.

**Simulation of $A$ and $E$, and estimation of the stomatal control coefficient**

In the summer season that is most important for carbon gain, weather in the Chequamegon National Forest is variable diurnally and interdielly. We chose a 19-day span from Julian days 204 through 222 in 1999 to represent the common mix of clear and cloudy conditions and of air temperatures and humidities that represent the passage of different air masses (synoptic highs, lows). We chose a thick canopy of leaf area index $= 6$, to represent limiting behavior in light interception. The real canopies fall somewhat short of this, in the lowland forest and the wetland, where LAI is nearer 4; this has little effect on performance. We chose parameter values $m = 10$, $b = 0.06$ mol m$^{-2}$s$^{-1}$, and $V_{c,max}^{25} = 100$ $\mu$mol m$^{-2}$s$^{-1}$. These are consistent with mean values of each parameter for upland forest overstory vegetation, and with the observed correlation of $b$ with $m$ in the upland forest.

Figure 5 presents the simulated assimilation and transpiration rates. It also presents the control coefficients for $g_s$ over both $A$ and $E$. These coefficients are defined (Kacser and Burns 1973; Stitt 1994) as the relative change in $A$ (or $E$) for a given relative change in $g_s$, 

-15-
\[ C_A = \frac{dA/A}{dg_s/g_s} \rightarrow \frac{d \ln(A)}{d \ln(g_s)}. \]  

We estimated these numerically by rerunning the simulation at each time interval with \( g_s \) increased by 10%. The calculations are omitted for times when some dewfall occurs, which is represented as \( E < 0 \) for some leaf area and which makes \( C_E \) rather meaningless numerically. We readily evaluate as well the control coefficients over time-integrated fluxes, \( A_{\text{int}} = \int A \, dt \) and \( E_{\text{int}} = \int E \, dt \). The simulation is not compared to tower fluxes or SiB2 simulations here, though the values may be close even with our neglect of understory and open-water fluxes. Our purpose is to estimate the effects of parameter variability and stomatal control.

The control coefficient is most significant for \( E \), as one expects. It ranges from 0.146 to 0.774 over the 19 days and has a mean value of 0.297. This magnitude of overall control implies that physiological control of \( E \) is important and that stomatal control must be represented accurately. This control emerges despite the relatively small canopy boundary-layer conductance, which is less than the effective canopy stomatal conductance (about one-half as large). It is also notable that stomatal control over \( E \) is weakest at times of peak transpiration and strongest at times of low \( E \) (vertical dotted lines in Fig. 5). In contrast, the stomatal control coefficient over \( A \) peaks simultaneously with \( A \) itself. The coefficient \( C_A \) is notably smaller (range 0.004 to 0.182; average 0.104), which is also expected from concise arguments about water-use efficiency at the leaf level (see, e.g., Jones 1992). Its small magnitude implies that physiological control is only modestly important for quantifying the forest C-sink...although an error of 8 to 10% in \( A_{\text{int}} \) readily changes a sink to a source (Goulden et al. 1996)!

We may also evaluate how important it is to represent key parts of the stomatal response to the environment. The model incorporates logical switches that allow the BB response to be shut off piecewise - responses to \( h_s \), to \( A \), or to both can be eliminated, with \( h_s \) or \( A \) replaced by fixed, mean values. A relevant comparison is the calculation of \( A_{\text{int}} \) for the full BB response vs. the case of no \( h_s \) response, adjusting the slope \( m \) and intercept \( b \) to get the same total water use, \( E_{\text{int}} \). Without the \( h_s \) response, \( A_{\text{int}} \) decreases by 1.0 %. This does not imply that the response to humidity is unimportant. The response to diurnal variations is not so significant, but response to long-term average humidity is important, as one sees by simulations with varied average
humidity, or with varied Ball-Berry slope \( m \); changing \( m \) by 10\% changes both \( A_{\text{int}} \) (+3.7\%) and \( E_{\text{int}} \) (+1.4\%), thus changing water-use efficiency \( A/E \) by +2.3\%. In simulations without the Ball-Berry responses to both \( h_s \) and to \( A \), \( A_{\text{int}} \) drops by a significant 6.2\%. These changes in water-use efficiency are consistent with the optimization theory of stomatal control (Cowan and Farquhar 1977). Overall stomatal control remains significant. The effective control coefficient of \( g_s \) over carbon flux is then 0.143 and over water flux it is 0.324. Removing both the \( h_s \) and \( A \) responses reduces the stomatal model almost to a Penman-Monteith style of model (Monteith 1963), with a single prescribed daytime conductance independent of other environmental conditions. (The model does retain a difference in effective sunlit leaf area, however, so that canopy conductance changes with solar elevation.) Intriguingly, the importance of the responses to \( h_s \) and to \( A \) is not seen in a simpler model of stand fluxes that has all the elements described here except that only one, averaged leaf irradiance is used rather than distributions of irradiance. In this model, turning off the \( h_s \) and \( A \) responses while enforcing the same water use \( (E_{\text{int}}) \) changes \( A_{\text{int}} \) negligibly, by 0.1\%!

**Estimation of the importance of physiological parameter accuracy**

The calculation of control coefficients is illuminating but it does not resolve the importance of individual physiological parameters. For studying the latter, we reran the model with a wide variety of choices of \( m \) and of \( V_{\text{c,max}}^{25} \). We fixed \( b \) by its correlation with \( m \); this both represents physiological reality and allows the results for \( A_{\text{int}} \) or \( E_{\text{int}} \) to be presented in three-dimensional plots.

Figure 6 presents simulations with \( m \) varied over the full range of 5 to 11 observed in the upland forest. Similarly, \( V_{\text{c,max}}^{25} \) is varied from 60 to 115 \( \mu \text{mol m}^{-2} \text{s}^{-1} \). A striking feature is that the carbon flux, \( A_{\text{int}} \), is virtually insensitive to the value of \( m \) over most of this range. (There are, however, declines at the highest values of \( m \), related to occurrence of \( b < 0 \) and consequent periods of strong stomatal closure.) Moreover, carbon flux is highly linear in its response to \( V_{\text{c,max}}^{25} \), especially for \( m < 10 \) (which obviates \( b < 0 \)). Neither of these features are apparent in simulations of single leaves in single environments, allowing independent variation of \( b \) (results not
shown; they are too diverse). We did additional simulations to discern the cause of this insensitivity to $m$ and this linearity in $V_{c,max}^{25}$. Use of a single environment (one day, at one time of day) did not alter the results. Use of a single, averaged irradiance similarly did not affect results. However, eliminating the correlation of $b$ with $m$ and holding $b$ fixed made a remarkable difference. A low value of $b$, 0.01 mol m$^{-2}$s$^{-1}$, introduced marked sensitivity of $A_{\text{int}}$ to $m$ and made the response nonlinear (Fig. 6c). A value of $b = 0.06$, which is consistent with the mean value (and with the $m$ - $b$ correlation at mean $m = 8$) diluted the $m$-sensitivity and the $V_{c,max}^{25}$-linearity. For control of integrated water use, $E_{\text{int}}$, we see that increasing $m$ actually decreases water use. The decrease in intercept $b$ is more important than the direct effect of the rise in $m$. Increasing $V_{c,max}^{25}$ does increase $E_{\text{int}}$ significantly. (Again, at high $m$, there are phenomena related to intercept $b < 0$ and consequent strong stomatal closure.) Table 4 summarizes these results.
Conclusions

Physiological control of leaf and stand fluxes appears to be adequately expressed using the slope $m$ and the intercept $b$ of the Ball-Berry model of stomatal control and the carboxylation capacity $V_{c,max}^{25}$. Additional control by downregulation of electron-transport capacity at high irradiance and of $V_{c,max}^{25}$ by high temperature appears not to be important. All three parameters, $m$, $b$ and $V_{c,max}^{25}$, show large variation, only some of which is explained by site, species, or season.

The implications of the residual variabilities for accurate modelling of fluxes have been explored with a big-leaf model for the dominant overstory fluxes. It appears to be important to account for the distribution of leaf irradiances that arise from leaf orientation and depth in the canopy, as well as for interception of thermal infrared flux. The response of stomata to humidity appears to be important more as a long-term mean rather than to details of the daily course of humidity. This finding is unexpected and derives from the stomatal control coefficients for $A$ and for $E$ peaking exactly out of phase. In turn, this derives in part from representing stomatal behavior with the Ball-Berry model, which is nonetheless an accurate representation of our experimental data. Also, the importance of stomatal responses to humidity and to photosynthetic rate are not reproduced in simpler simulation models, including SiB2, that use a single average irradiance on leaves rather than distributions of irradiance that account for varied leaf orientations and depths in the canopy.

Errors in modelling $g_s$ propagate significantly to integrated water flux, $E_{int}$ because the control coefficient from $g_s$ to $E_{int}$ is large (about 0.35). Errors propagate weakly to the integrated CO$_2$ flux $A_{int}$ (control coefficient about 0.1), but are magnified again in the net C-sink, which is on the order of one-tenth of $A_{int}$. The errors in $E_{int}$ should affect $A_{int}$ indirectly via the generation of water stress; this should be important only intermittently, as observed in the field.

Modelled carbon and water fluxes are remarkably linear in the parameters $m$ and $V_{c,max}^{25}$ when one accounts for the dependent behavior of $b$ upon $m$. The slope of this relation appears to be almost exactly that needed to make carbon flux almost insensitive to $m$. The negative correlation of $b$ with $m$ is expected to be common, but it is unknown if the slope of this relation is commonly in the range to make $A_{int}$ insensitive to $m$; measurements in other ecosystems are merited.
Neither can we estimate if this relationship is closely regulated by natural selection. At least in the Chequamegon National Forest, it appears valid to use detailed flux simulations with single, averaged values of the parameters $m$, $b$ and $V_{c,\text{max}}^{25}$, in view of the fluxes’ linearity in these parameters. The most important of the parameters for flux modelling appears to be $V_{c,\text{max}}^{25}$; $V_{c,\text{max}}^{25}$ is a strong determinant of assimilation directly, and of stomatal conductance and transpiration indirectly via the empirical scaling of $g_s$ to $A$. The parameter values in the SiB2 database for the site appear in need of minor revision, such as changing $m$ from 10 to 8.

In modelling $g_s$, one may need to use negative values of the Ball-Berry intercept that repeatedly occur in analyses of field data. These negative intercepts require special versions of programs that simultaneously solve the Ball-Berry, assimilation, and energy balance equations. We will freely provide such programs, coded in Fortran 77 for portability.

Our field data do not yet suffice to predict $V_{c,\text{max}}^{25}$ as a function of vegetation type, canopy position, and seasonal environment. A priority for future research should be field studies to quantify the patterns and tests of models of such acclimation (e.g., Niinemets et al. 1998b). The inclusion of water-stress effects on $g_s$ and $V_{c,\text{max}}^{25}$ is also important. A first step is to discern how the responses are composed from separate signals on leaf and root water status, such as are seen in herbaceous plants (Tardieu and Davies, 1993; Tardieu and Simonneau, 1998). Tall trees may act similarly (Niinemets et al. 1999), while in other cases have been shown to have more mixed responses to abscisic acid and hydraulic signals (Auge et al. 2000; Saliendra et al. 1995) or to have abscisic acid flux as unresponsive to water stress and thus unlikely to be a stress signal (Triboulot et al. 1996). The more complex signalling may be need for effective control of water status when there are long transport delays in hormonal signalling from roots to leaves.

We did not explore the effects of structural features of the canopy upon modelled fluxes. Effects of leaf clumping might be significant and might be tested in specialized models such as ours, using simple corrections to light interception (e.g., Gutschick 1991). Our results support the current methods of flux simulations with comprehensive models such as SiB2. These modelling efforts will reveal if physiological control has other important interactions, particularly with the CO$_2$ rectifier effect (Denning et al. 1995, 1999).
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