Oliver, Rebecca J.; Taylor, Gail; Finch, Jon W.. 2012 Assessing the impact of internal conductance to CO2 in a land-surface scheme: measurement and modelling of photosynthesis in Populus nigra. *Agricultural and Forest Meteorology*, 152. 240-251. [10.1016/j.agrformet.2011.10.001](http://nora.nerc.ac.uk/15782/)

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Assessing the impact of internal conductance to CO₂ in a land-surface scheme:
Measurement and modelling of photosynthesis in *Populus nigra*

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Abbreviations:

Atmospheric CO₂ concentration (c_a); intercellular CO₂ concentration (c_i); chloroplastic CO₂ concentration (c_c); net photosynthesis (A_{net}); stomatal conductance (g_s); maximum carboxylation rate of Rubisco (V_{max}); maximum rate of electron transport (J_{max}); internal CO₂ conductance (g_{i}); intrinsic quantum efficiency (α_{int}); apparent quantum efficiency (α_{app}); c_i/c_a ratio for specific humidity deficit in the canopy (F_0); critical humidity deficit (D_c)

Abstract

Vegetation plays a key role in both the global carbon and water cycles. Therefore, the representation of leaf-level fluxes of carbon and water in process-based land-surface schemes is central to accurately predicting these surface exchanges on a larger scale. Leaf-level models of photosynthesis used in such schemes are commonly based on the equations of Farquhar *et al.*, (1980), which were founded on the assumption that differences in the drawdown of CO₂ from sub-stomatal cavities (c_i) to the site of carboxylation inside chloroplasts (c_c) were negligible. Recent research, however, indicates an important role for this additional internal pathway of CO₂ transfer (g_i) in photosynthesis. This work therefore combined fieldwork and modelling to assess the impact of g_i on estimation of key photosynthetic parameters, and on the accuracy of simulated photosynthesis (A_{net}) and stomatal conductance (g_s) in a coupled model of leaf-level A_{net} and g_s embedded in a land-surface scheme. It was shown that, in a fast growing poplar genotype (*Populus nigra*), the photosynthetic parameter V_{max} was sensitive to g_i. Determination of V_{max} under the assumption of finite g_i led to estimates of V_{max} in well-watered trees that were, on average, 52 % higher than values calculated on a c_i basis. Drought induced declines in all key photosynthetic parameters measured were observed (V_{max}, J_{max}
and $g_s$), in addition to a two-fold increase in photosynthetic biochemical capacity upon re-watering. Reasons for this and the implications for land-surface modelling are discussed. It was shown that inclusion of a constant (non-water stressed) internal conductance to CO$_2$ in a coupled model of leaf-level $A_{net}$ and $g_s$ did not improve the accuracy of these simulated fluxes. It was concluded that, for application within a land-surface scheme, currently, accurate calibration of $V_{max}$ potentially has a greater impact on simulated $A_{net}$ and $g_s$ than the inclusion of additional, fine-scale leaf-level processes such as $g_i$.

Keywords: land-surface model, poplar, gas exchange, $V_{max}$, mesophyll conductance

1. Introduction

From the gains and losses of carbon through photosynthesis and respiration, and the passage of water through plant stomata to the atmosphere, vegetation plays a major role in both the global carbon and water cycles. For land-surface schemes to correctly simulate carbon and water budgets, they must accurately represent the processes of carbon and water exchange from vegetated surfaces. Land-surface schemes commonly model carbon exchange of vegetation using biochemical models of leaf-level photosynthesis based on the equations of Farquhar et al., (1980), coupled to a stomatal conductance model to simulate leaf-level fluxes of carbon dioxide and water. These are then scaled up to simulate carbon and water exchanges at the canopy-level. Therefore, correct parameterisation of these models at the leaf-level is central to accurate predictions of vegetation productivity and water-use at the larger-scale. The work of Hughes et al., and Vanloocke et al., (2010) for example, both used land-surface schemes to determine the carbon- and water-balances respectively of extensive plantings of Miscanthus x giganteus, a C$_4$ perennial grass bioenergy crop. Used in applications such as these, it is imperative that models are parameterised appropriately, and that simplifications used to represent key processes in models are adequate.

Until recently, photosynthesis in plants was considered to be limited dominantly by two factors; $g_s$, which regulates the CO$_2$ supply into the leaf, and leaf biochemistry, which is the basic photochemistry, carboxylation and Calvin cycle reactions that regulate the CO$_2$ demand (Flexas et al., 2008). Consequently, models of photosynthesis, such as Farquhar et al., (1980) and Collatz et al., (1991; 1992), were founded on the assumption that differences in the CO$_2$ concentration in the substomatal cavities and at the site of carboxylation in the chloroplast stroma were negligible. In other words, $c_i$ (the intercellular CO$_2$ concentration) was equal to $c_c$ (the chloroplastic CO$_2$ concentration). Recent research, however, identified the important role of internal CO$_2$ conductance ($g_i$) in regulating
photosynthesis, i.e. the transfer of CO$_2$ across mesophyll cells from $c_i$ to $c_c$. There is increasing evidence suggesting that $g_i$ is actually finite and can itself respond to changing environmental conditions, such that it can impose a significant limitation on photosynthesis (Centritto et al., 2003; During, 2003; Flexas et al., 2007a; Flexas et al., 2002; Grassi and Magnani, 2005; Warren et al., 2004). Current research suggests that $g_i$ is of similar quantitative importance to stomata and Rubisco in terms of limiting/regulating photosynthesis (Ethier and Livingston, 2004; Flexas et al., 2008; Warren, 2008). Therefore, it is suggested that it may be necessary to re-formulate photosynthesis models to include this process in order to improve predictions of leaf-level carbon assimilation (Ethier and Livingston, 2004; Flexas et al., 2008; Niinemets et al., 2009).

The present study addresses two questions: (1) what is the impact of internal conductance to CO$_2$ ($g_i$) on estimates of the key photosynthetic parameters $V_{max}$ (the maximum rate of carboxylation at Rubisco) and $J_{max}$ (the maximum rate of electron transport) in *Populus nigra*? (2) does the inclusion of this additional pathway of CO$_2$ transfer in a coupled model of leaf-level photosynthesis – stomatal conductance improve the accuracy of these two simulated fluxes? CO$_2$- and light-response curves were measured on a variety of poplar (*P. nigra* L.) to determine the impact of $g_i$ on estimates of the key photosynthetic kinetic parameters. This data was used to test and calibrate a coupled model of leaf-level photosynthesis and stomatal conductance embedded in a land-surface scheme called JULES (Best et al., 2011; Clark et al., 2011). The leaf-level model was modified to include the transfer of $c_i$ to $c_c$ to assess the impact of $g_i$ on the accuracy of predicted photosynthesis and stomatal conductance. An independent data set was used to validate the performance of the different model configurations.

2. Materials and Methods

2.1. Plant material and experimental setup

Established cuttings of *P. nigra* L. (cv. Jean Pourtet) were cultivated at Wytham field station (Wytham, Oxfordshire, UK; 51°44’99”N, 1°18’97”W). In April 2008, the cuttings were potted into 10 L pots (300 mm diameter x 250 mm depth) using a soil-based, lime-free compost (John Innes No. 3). Fifty trees (25 per treatment) were arranged in a split-plot design. Trees were randomly distributed between four blocks. Two blocks were subject to periods when water was withheld to impose a drought treatment. The remaining two blocks were watered continuously over the course of the experiment. $A_{net}$ and $g_s$ were measured over the course of the experiment under ambient atmospheric conditions. Recordings were made from at least four, and up to ten trees per block, per treatment at each measurement period (before, during and after each drought period). Trees were
chosen at random, and measurements were made on the first fully expanded, sun-exposed leaf (i.e. one leaf per tree). Three recordings on the same leaf were made, and the average of these was used in analyses. Measurement of response curves used three trees per treatment, and the same trees were used over the course of the experiment. Trees had been selected at random from the blocks, and curves were measured on the first fully expanded leaf of each tree.

Before the onset of experiments all trees were fully watered. Pots were spaced at 300 mm intervals to avoid shading and allow access to the trees. Watering treatments began when leaves were completely developed and matured. Control trees were continuously watered so their soil moisture content remained near to field capacity (around 30% volume). For the duration of the experimental period, mean soil moisture of control trees was 30.6% vol. ± 3.5% vol. Stressed trees endured two periods of imposed soil water stress where they were not watered and pots were shielded from rainfall by the use of a polythene cone fitted around the base of the stem and the lip of the pot so the canopy remained exposed to the atmosphere. The protective covers could be raised and lowered as necessary to allow circulation of air beneath during dry periods. Any effect of the use of these covers on soil temperature was deemed minimal when compared to the effect of reduced moisture content on soil temperature. In total, water was withheld for 25 days (2 to 26 June) during the first drought cycle; trees were then fully re-watered for eight weeks until the onset of the second drought cycle, which lasted 40 days (20 August to 28 September). In both drought cycles, plants were kept without water until net photosynthesis was almost completely inhibited during the late morning.

2.2. Soil moisture

Soil moisture content was monitored continuously over the course of the experiment using SM200 soil moisture sensors (Delta-T Devices Ltd, Cambridge, UK). Soil moisture was recorded as % volumetric water content (% vol.). Twelve sensors in total were used, so soil moisture content of six trees per treatment could be continuously logged. Point measurements were also made to check the soil moisture content of pots without sensors.

2.3. Leaf-gas exchange measurements

Leaf-level gas exchange was recorded using a portable infrared gas exchange analyser (IRGA) system (CIRAS-2, PP-systems, Hitchin, UK). For all measurements, the leaf area used was 250 mm². *P. nigra* is amphistomatous so the stomatal ratio was maintained at 30% for the upper- and 70% for...
the lower-leaf surface. This ratio had been determined from previous measurements of the
correlation of stomata on the abaxial and adaxial leaf surfaces to the rate of \( g_s \) in this genotype
(Ingmar Tulva <i>pers. comm.</i>, 2007).

2.3. Leaf-gas exchange under ambient atmospheric conditions

Leaf-level \( A_{\text{net}} \) and \( g_s \) were measured in situ under ambient atmospheric conditions over the course of
the experiment. Measurements were made during the hours 09:00-12:00 GMT, and were restricted to
days with clear skies; temperature and relative humidity inside the leaf chamber were close to
ambient values. The \( \text{CO}_2 \) concentration inside the leaf chamber was maintained at \( 380 \pm 5 \text{ ppm} \) using
a \( \text{CO}_2 \) cartridge plugged into the CIRAS-2.

2.4. \( \text{CO}_2 \) response curve

The response of \( A_{\text{net}} \) to increasing concentrations of \( c_i \) was measured in situ using the IRGA system.
Measurements were made between the hours of 09:00 and 14:00 GMT. Leaf temperatures were set at
25 °C for all measurements, leaves were illuminated using a red-blue LED light source attached to
the gas exchange system and photosynthetic photon flux density (\( P_{\text{PFD}} \)) was maintained at 1500 \( \mu \text{mol} \)
\( \text{m}^{-2} \text{s}^{-1} \). According to Bernacchi et al., (2003) this level of \( P_{\text{PFD}} \) is just above the light saturation point
for this species. Leaf vapour pressure deficits were maintained close to ambient. Following protocols
suggested by Long and Bernacchi (2003) and Bernacchi et al., (2003), leaves were incubated at a
\( \text{CO}_2 \) concentration of 200 ppm for 20-30 minutes prior to measurement to maximise stomatal
opening. Measurement of \( A_{\text{net}} - c_i \) curves followed the method of Bernacchi et al. (2003) starting at
400 ppm \( \text{CO}_2 \), decreasing stepwise to 50 ppm, then increasing stepwise to 1800 ppm \( \text{CO}_2 \).

The \( A_{\text{net}} - c_i \) curves were fitted using the method of Sharkey et al., (2007) to provide optimised
estimates of \( g_i \) (\( \mu \text{mol} \text{ m}^{-2} \text{s}^{-1} \text{ Pa}^{-1} \)), \( V_{\text{max}} \) (\( \mu \text{mol} \text{ CO}_2 \text{ m}^{-2} \text{s}^{-1} \)) and \( J_{\text{max}} \) (\( \mu \text{mol} \text{ electrons m}^{-2} \text{s}^{-1} \)). An online
analytical tool to aid with curve fitting can be found at:
www.blackwellpublishing.com/plantsci/pcecalculation. This method uses the biochemically based
model for photosynthesis of Farquhar et al., (1980) with modifications for finite internal \( \text{CO}_2 \) transfer
(\( g_i \)), which uses \( c_c \) instead of \( c_i \) where \( c_c = c_i - A/g_i \). This model was then adapted to calculate \( V_{\text{max}} \) and
\( J_{\text{max}} \) at \( c_i \), where \( c_c = c_i \). For more information see Sharkey et al., (2007) and Pons et al., (2009).

2.5. Light response curve
Leaves were sampled as described for $A_{\text{net}} - c_i$ measurements above. Leaves were placed in the leaf chamber and illuminated until steady-state rates of $A_{\text{net}}$ and $g_s$ had been achieved. Leaf temperature was set at 25 °C for all measurements and CO$_2$ concentration was maintained at 380 ppm. $A_{\text{net}} - P_{PFD}$ response curves were then measured starting at saturating light (2000 µmol m$^{-2}$ s$^{-1}$) and decreased stepwise to darkness.

The $A_{\text{net}} - P_{PFD}$ response curves were analysed using the software ‘Photosynthesis Assistant’ (Parsons and Ogston, 1998), which uses the equation given by Prioul & Chartier (1977). The software fits the equation through an iterative process to give parameter values associated with the smallest error.

2.6. Models

2.6.1. The coupled model for leaf-level photosynthesis and stomatal conductance

The photosynthesis – stomatal conductance model used in this work is embedded in the land-surface scheme JULES (Best et al., 2011; Clark et al., 2011), hereafter referred to as the JULES $A_{\text{net}} - g_s$ model. This sub-model calculates the leaf-level exchanges of carbon and water. These are described as being dependent on a number of environmental variables as well as $c_i$, with an additional direct dependence on soil moisture status. This sub-model is based on the photosynthesis model of Collatz et al., (1991) for C$_3$ plants and Collatz et al., (1992) for C$_4$ plants, and uses the stomatal closure described by Jacobs (1994).

2.6.2. Overview of the modelling

The measured $A_{\text{net}} - c_i$ and $A_{\text{net}} - P_{PFD}$ response curves provided parameter values for $P$. nigra to test and calibrate the JULES $A_{\text{net}} - g_s$ model. The JULES $A_{\text{net}} - g_s$ model was used in the following configurations; 1) the original configuration 2) modified to include the transfer of CO$_2$ from $c_i$ to $c_s$, and 3) modified to use the photosynthesis model of Farquhar et al., (1980) and include internal CO$_2$ conductance. The accuracy of simulated $A_{\text{net}}$ and $g_s$ was compared in these three different model configurations. Model testing, calibration and validation occurred in three steps:

i) The performance of each model configuration was tested after being parameterised with individual values for the photosynthetic parameters taken from separate $A_{\text{net}} - c_i$ and $A_{\text{net}} - P_{PFD}$ response curves.

The accuracy of simulated $A_{\text{net}}$ in response to increasing concentrations of atmospheric CO$_2$ was compared against the measured $A_{\text{net}} - c_i$ response curves.

ii) When used in the land-surface scheme, the coupled $A_{\text{net}} - g_s$ model requires a single value for each of the photosynthetic parameters. Therefore, the average value of each photosynthetic parameter
derived from the $A_{net} - c_i$ and $A_{net} - P_{PFD}$ response curves, measured in well-watered trees, was used to calibrate the model, and model performance was assessed again. Model performance was also assessed using a default set of model parameter values as opposed to calibrated values.

ii) Using the calibration performed in step ii), the model configurations were validated against an independent data set of leaf-level $A_{net}$ and $g_s$, measured across the growing season under ambient atmospheric conditions, in healthy and water stressed top of canopy leaves of *P. nigra*.

### 2.6.3. Model configurations

The three different configurations of the JULES $A_{net} - g_s$ model are summarised in Table 1. Model 1 is the photosynthesis sub-model currently used in JULES. Model 2 is equivalent to Model 1, but the transfer of $c_i$ to $c_c$ has been included according to Ethier & Livingston (2004). Model 3 uses the configuration of the Farquhar *et al.*, (1980) photosynthesis model, which has been modified in the same manner as Model 2 to include the transfer of $c_i$ to $c_c$. The main differences between the basic configuration of the Collatz *et al.*, (1991) model (Model 1 and Model 2) and the Farquhar *et al.*, (1980) model (Model 3) is the description of the dependence of photosynthetic rate on light. The Farquhar *et al.*, (1980) model uses an additional parameter, $J_{max}$, to determine the light limited rate of photosynthesis, whereas Model 1 and Model 2 use the Collatz *et al.*, (1991) dependence on quantum yield. Model 2 and Model 3 use exponential temperature response functions for key temperature dependent parameters; $K_o$ (Michaelis-Menton constant of Rubisco for O$_2$), $K_c$ (Michaelis-Menton constant of Rubisco for CO$_2$), $I^*$ (chloroplastic CO$_2$ photocompensation point in the absence of mitochondrial respiration), $V_{max}$, $J_{max}$, $R_d$ (dark respiration) and $g_i$. The temperature response functions used in Model 2 and Model 3 are those shown in Sharkey *et al.*, (2007) and are reproduced here in equations 8 and 9. The Rubisco kinetic constants ($K_o$, $K_c$, $I^*$) used in Model 2 and Model 3 are taken from Sharkey *et al.*, (2007) and have been determined *in vivo* at $c_c$ (Table 2). Because both Model 2 and Model 3 include $g_i$, they were parameterised with values of $V_{max}$ estimated at $c_c$ instead of $c_i$.

Model 1 and uses $Q_{10}$ temperature response functions as shown in Collatz *et al.*, (1991) (see equation 7; Table 2). For all three models, calculation of the dark respiration rate and the triose-phosphate export limited rate of photosynthesis were the same, and followed the approach used in the Collatz *et al.*, (1991) model.

### 2.6.4. Modelling photosynthesis with internal conductance to CO$_2$

Models 2 and 3 were modified to include the transfer of CO$_2$ from intercellular air spaces across the mesophyll cell wall and into the chloroplast. This followed the procedure of Ethier & Livingston (2004) who modified the biochemically based photosynthesis model of Farquhar *et al.*, (1980) to
include this transfer. They developed a non-rectangular hyperbola version of the model that includes
$g_i$ to calculate both the CO$_2$- and light-limited rates of photosynthesis at the CO$_2$ concentration inside
the chloroplast ($c_c$). This approach was taken in both Model 2 and Model 3. Under Rubisco limited
conditions, the rate of photosynthesis can be determined at $c_c$ by equation 1:

$$W_{\text{carbc}} = \frac{(c_c - \Gamma^*)V_{\text{max}}}{c_c + K_c (1 + O_a / K_o)} - R_d$$  \hspace{1cm} (1)$$

Where, $W_{\text{carbc}}$ (µmol CO$_2$ m$^{-2}$ s$^{-1}$) is the CO$_2$- limited (or RuBP - saturated) CO$_2$ assimilation rate
determined at $c_c$, $c_c$ (Pa) is the chloroplastic CO$_2$ concentration and $\Gamma^*$ (Pa) is the chloroplastic CO$_2$
photocompensation point in the absence of mitochondrial respiration. Substituting $c_c$ with equation 2,
where $g_i$ (µmol CO$_2$ m$^{-2}$ s$^{-1}$ Pa$^{-1}$) is the internal CO$_2$ conductance transfer, gives a quadratic equation
whose solution is the positive root (equation 3)

$$c_c = c_i - \frac{W_{\text{carbc}}}{g_i}$$  \hspace{1cm} (2)$$

$$W_{\text{carbc}} = \frac{-b + \sqrt{b^2 - 4ac}}{2a}$$

where,

$$a = -1 / g_i$$

$$b = (V_{\text{max}} - R_d) / g_i + c_i + K_c (1 + O_a / K_o)$$

$$c = R_d (c_i + K_c (1 + O_a / K_o)) - V_{\text{max}} (c_i - \Gamma^*)$$

$$\text{(3)}$$

The light-limited rate of photosynthesis at $c_c$, $W_{\text{litec}}$, can be derived in a similar manner. Using the
Farquhar et al., 1980 model (Model 3), $W_{\text{litec}}$ is determined following equation 4:

$$W_{\text{litec}} = \frac{J / 4(c_c - \Gamma^*)}{c_c + 2\Gamma^*} - R_d$$  \hspace{1cm} (4)$$

Where, $J$ (µmol e$^{-}$ m$^{-2}$ s$^{-1}$) is the rate of electron transport dependent on irradiance ($I_{\text{par}}$), given in
equation 5 after Harley et al., 1992:

$$J = \frac{\alpha_{\text{app}} I_{\text{par}}}{\sqrt{1 + (\alpha_{\text{app}} I_{\text{par}} / J_{\text{max}})^2}}$$  \hspace{1cm} (5)$$
Where, $J_{\text{max}}$ (µmol e⁻ m⁻² s⁻¹) is the maximum electron transport rate, $\alpha_{\text{app}}$ (mol e⁻/mol photons⁻¹) is the apparent quantum efficiency, and $I_{\text{par}}$ (µmol m⁻² s⁻¹) is the photosynthetically active radiation. In the Collatz et al., (1991) model, the description of the dependence of photosynthetic rate on light is dependent on the quantum yield, and is shown in equation 6 for determination at $c_c$:

$$W_{\text{litec}} = \frac{\alpha_{\text{int}} \omega I_{\text{par}} (c_c - \Gamma^*)}{c_c + 2\Gamma^*} - R_d$$  

(6)

Where, $\omega$ is the leaf scattering coefficient for PAR (0.15) and $\alpha_{\text{int}}$ is the intrinsic quantum efficiency (mol CO₂ / mol⁻¹ PAR). For simplicity we will call the first three terms on the top of equation 6 $J_{\text{collatz}}$, which describes the dependency of photosynthetic rate on available light in the Collatz et al., (1991) model. Consequently, like $W_{\text{carbc}}$, the light-limited CO₂ assimilation rate, $W_{\text{litec}}$, can be derived as outlined in equations 1 to 3 for Model 2 and Model 3 by replacing $V_{\text{max}}$ with $J_{\text{collatz}}$ (Model 2) or with $J/4$ (Model 3), and $K_c(1 + O_a/K_o)$ with $2\Gamma^*$. The factor of four used in the Farquhar et al., (1980) model accounts for four electrons being required per carboxylation/oxygenation reaction.

2.6.5. Adjusting for temperature

In Model 1, temperature dependencies of the following parameters, $R_d$, $V_{\text{max}}$, $g_i$, $K_o$, $K_c$ and $\Gamma^*$ are described using a $Q_{10}$ function shown in equation 7 (see Table 2).

$$f_T = f_{T25}Q_{10}^{0.40(T_r - 25)}$$  

(7)

Where, $f_{T25}$ is the parameter value at 25 °C and $Q_{10}$ is the relative change in the parameter for a 10 °C change in temperature (Collatz et al., 1991). Exponential temperature response functions were used in Model 2 and Model 3. In this version of the model, the temperature dependence of $V_{\text{max}}$, $J_{\text{max}}$, $R_d$, $K_c$, $K_o$ and $\Gamma^*$ were each described by equation 8, (Harley et al., 1992; Sharkey et al., 2007):

$$Parameter = \exp(c - \Delta H_a / RT_c)$$  

(8)

Where, $c$ is a scaling constant, $\Delta H_a$ is an enthalpy of activation, $R$ is the perfect gas constant (8.314 J mol⁻¹ K⁻¹) and $T_c$ is the leaf absolute temperature (°C). The temperature dependence for $g_i$ used in Model 2 and Model 3 follows Bernacchi et al., (2002), and shown in equation 9 below:
Where, $\Delta H_d$ is an enthalpy of deactivation and $\Delta S$ is an entropy term. The parameter values used are shown in Table 2.

2.7. Data

2.7.1. Model testing

The key photosynthetic parameters required by the three model configurations are shown in Table 1. Individual values of $V_{\text{max}}$, $J_{\text{max}}$ and $g_i$ inferred from the measured $A_{\text{net}} - c_i$ response curves were used to test the performance of the three model configurations (Table 3). $V_{\text{max}}$ estimated without $g_i$ (i.e. at $c_i$), was used in Model 1, whereas $V_{\text{max}}$ estimated with $g_i$ was used in Models 2 and 3. Additionally, Model 3 used values of $J_{\text{max}}$ determined at $c_c$. The apparent quantum efficiency ($\alpha_{\text{app}}$) describes the efficiency of light utilization in photosynthesis, and was inferred from the measured $A_{\text{net}} - P_{\text{PFD}}$ response curves. The intrinsic quantum efficiency ($\alpha_{\text{int}}$), is similar to $\alpha_{\text{app}}$ but takes into account reflected and transmitted light and is therefore thought to be highly conserved across C3 species (Long et al., 1993). Typical values for $\alpha_{\text{int}}$ range between 0.06 to 0.125 mol CO$_2$ mol$^{-1}$PAR (Collatz et al., 1991; Farquhar et al., 1980; Laisk et al., 2002; Lambers et al., 2008; Long et al., 1993). It is also suggested that there is a close relationship between $\alpha_{\text{int}}$ and $F_v/F_m$ (the maximum potential quantum efficiency of photosystem II) as measured by chlorophyll fluorescence (Kao and Forseth, 1992). $F_v/F_m$ was also measured in well-watered $P$. nigra trees over the same experimental period. Individual results are not reported, but $F_v/F_m$ varied little over the course of the growing season, ranging between 0.072 - 0.08 mol CO$_2$ mol$^{-1}$ PAR. The average of this range (0.076 mol CO$_2$ mol$^{-1}$PAR) was chosen as the value of $\alpha_{\text{int}}$ to use in both Model 1 and Model 2. $F_0$ (unit-less) and $D_c$ (kg kg$^{-1}$) are additional model plant-specific parameters. $F_0$ is the $c/c_a$ for specific humidity deficit in the canopy, and $D_c$ is the critical humidity deficit. These parameters are not as readily measured, so it was necessary to tune these parameters to find suitable values. Each model was run in a Monte-Carlo simulation for 5000 iterations to find the parameter value that minimised the RMSD (root mean squared deviation) between modelled and observed $A_{\text{net}}$. The values of $F_0$ and $D_c$ that minimised the RMSD were close to the default values for C3 plants specified in the JULES documentation (Cox, 2001). It was observed that there was minimal sensitivity of simulated $A_{\text{net}}$ to each of these parameters. The same values were used in all model configurations. At this stage, model testing was only performed with parameters from well watered trees, therefore the soil moisture stress factor ($\beta$) in the model was set to one (i.e. no soil moisture stress), (Cox et al., 1998).
2.7.2. Model Calibration

The leaf-level $A_{net} - g_s$ model tested is integral to a land-surface scheme. This requires just one value for each parameter ($V_{max}$, $J_{max}$, $g_t$, $\alpha$, $F_0$ and $D_c$). Therefore, the mean across the growing season from well-watered trees was used for each parameter. These values are shown in bold at the bottom of Table 3. Model 1 was also used with a default parameter set for a broadleaf tree plant functional type (PFT) in the JULES model (JULES_def; $V_{max} = 32.00$, $\alpha_{int} = 0.076$, $F_0 = 0.875$, $D_c = 0.09$). This was to assess the accuracy of modelled $A_{net}$ when using default parameter values as opposed to calibrated parameters. This could not be done to compare the performance of Model 2 or Model 3 as default values for $J_{max}$ and $g_t$ were unknown.

2.7.3. Model Validation

Model validation was performed against measured leaf-level rates of $A_{net}$ and $g_s$ across the growing season in *P. nigra* trees under ambient environmental conditions and with imposed soil moisture stress. The soil moisture stress factor was calculated according to Cox *et al.*, (1998).

2.7.4. Model assessment and statistical analyses

Observed data were plotted against their corresponding model-predicted values, model bias and goodness of fit was assessed based on the intercept, slope and coefficient of determination ($r^2$) of the optimal least squares regression line. Based on the recommendation of Piñeiro *et al.*, (2008) all model assessments used regressions of observed (in the y-axis) vs. predicted (in the x-axis). The root mean squared deviation (RMSD) was also estimated.

Statistical analysis used a linear mixed effects model with the package *lme4* (Bates and Maechler, 2009) available in the statistical software *R2.10.1* (R2.10.1, 2009). The analysis tested main effects and interactions, with the random effect of block nested within treatment levels, and day of year. Significance of fixed effects were tested for using likelihood ratio tests (LRT) that use the chi-squared ($x^2$) distribution and maximum likelihood estimators (MLE) (Faraway, 2006).

2.7.5. Model driving data

Required meteorological driving variables were; leaf temperature (K), air pressure (Pa), PAR (W m$^{-2}$) and specific humidity ($Q$; kg kg$^{-1}$). Leaf temperature, air pressure and PAR were measured and
recorded by the IRGA at the time of measurement. Specific humidity \(Q\) (kg kg\(^{-1}\)) was calculated using equation 10, where \(e_a\) is the actual vapour pressure (kPa) as measured by the IRGA:

\[
Q = 0.622e_a^{0.01}
\]

(10)

3. Results

3.1. Photosynthetic parameters

An effect of \(g_i\) on the estimate of \(V_{\text{max}}\) was detected in both well-watered and stressed trees \((x^2 = 15.7, p < 0.01, n = 50)\). Estimates of \(V_{\text{max}}\) made assuming finite \(g_i\) resulted in significantly higher values of \(V_{\text{max}}\) (Fig 1a & b). Over the course of the growing season, in well-watered trees, \(V_{\text{max}}\) estimated at \(c_c\) ranged from 89.95 ± 21.7 µmol CO\(_2\) m\(^{-2}\) s\(^{-1}\) to 106.84 ± 24.6 µmol CO\(_2\) m\(^{-2}\) s\(^{-1}\) depending on measurement period, however, estimated at \(c_i\), \(V_{\text{max}}\) ranged between 58.51 ± 12.2 µmol CO\(_2\) m\(^{-2}\) s\(^{-1}\) to 70.64 ± 21.9 µmol CO\(_2\) m\(^{-2}\) s\(^{-1}\). In either case, the estimated values of \(V_{\text{max}}\) remained fairly constant for the duration of the growing season in control trees, with measurements falling well within the error of each other (Fig. 1a & b). During the measurement periods preceding a drought (Predrt.1 and Predrt.2 in Fig. 1), \(V_{\text{max}}\) estimated in both control and treatment trees were similar, falling well within the measurement error of each other (Fig. 1a & b). Drought led to a substantial decrease in estimated \(V_{\text{max}}\) compared to well-watered trees \((x^2 = 11.78, p < 0.01, n = 50\); Fig 1a & b). The mean of \(V_{\text{max}}\) estimated for stressed trees during the first drought period was 29.88 ± 13.7 µmol CO\(_2\) m\(^{-2}\) s\(^{-1}\) \((106.42 ± 4.9 µmol CO_2 m^-2 s^-1)\) and 20.80 ± 5.8 µmol CO\(_2\) m\(^{-2}\) s\(^{-1}\) \((69.97 ± 5.9 µmol CO_2 m^-2 s^-1)\) with and without \(g_i\) respectively (numbers in brackets indicate the corresponding value measured in control trees). During the second drought period this was 42.39 ± 3.9 µmol CO\(_2\) m\(^{-2}\) s\(^{-1}\) \((94.32 ± 15.4 µmol CO_2 m^-2 s^-1)\) and 39.81 ± 2.9 µmol CO\(_2\) m\(^{-2}\) s\(^{-1}\) \((68.04 ± 3.8 µmol CO_2 m^-2 s^-1)\) respectively. Mean soil moisture content during the first drought period (i.e. reported from the period when water was first withheld to just before re-watering) was 8.8 ± 6.8 % vol. with a minimum of 1.5 % vol., during the second drought period mean soil moisture content was 19.3 ± 7.9 % vol. with a minimum of 3.5 % vol (Fig. 2). It is notoriously difficult to impose a controlled drought, nevertheless, soil moisture content during both drought periods was sufficiently reduced to impact on estimates of \(V_{\text{max}}\) measured at both \(c_i\) and \(c_c\) (Fig. 2b). During the recovery period, \(V_{\text{max}}\) estimated in trees that were re-watered following drought was substantially higher than the control trees (Rec. in Fig. 1a & b). \(V_{\text{max}}\) estimated at \(c_c\) in trees recovering from drought was 230.14 ± 28.0 µmol CO\(_2\) m\(^{-2}\) s\(^{-1}\) compared to 89.95 ± 21.7 µmol CO\(_2\) m\(^{-2}\) s\(^{-1}\) measured in control trees.
There was no detectable effect of $g_i$ on the estimate of $J_{\text{max}}$. As seen in Fig. 1c & d, estimates of $J_{\text{max}}$
both with and without $g_i$ were similar, for control trees these ranged between 121.84 ± 12.4 µmol CO$_2$ m$^{-2}$ s$^{-1}$ and 156.84 ± 48.7 µmol CO$_2$ m$^{-2}$ s$^{-1}$ when measured at $c_c$, and 108.18 ± 14.7 µmol CO$_2$
m$^{-2}$ s$^{-1}$ to 145.99 ± 50.0 µmol CO$_2$ m$^{-2}$ s$^{-1}$ when measured at $c_p$. Drought caused a significant decline
of $J_{\text{max}}$ ($x^2=12.70, p < 0.01, n = 50$). $J_{\text{max}}$ in drought stressed trees declined to 47.87 ± 18.2 µmol CO$_2$
m$^{-2}$ s$^{-1}$ with $g_i$ and 38.55 ± 11.4 µmol CO$_2$ m$^{-2}$ s$^{-1}$ without $g_i$ during the first drought period, and
53.35 ± 5.3 µmol CO$_2$ m$^{-2}$ s$^{-1}$ and 51.81 ± 4.5 µmol CO$_2$ m$^{-2}$ s$^{-1}$ respectively during the second
drought period. Similar to $V_{\text{max}}$, it is likely that $J_{\text{max}}$ declined less during the second drought period
because the reduction is soil moisture content was less severe (Fig. 2). As stressed trees were re-
watered following the first drought, $J_{\text{max}}$ measured in these trees was significantly higher compared
to control trees. In stressed trees recovering from drought, $J_{\text{max}}$ was 260.21 ± 14.0 µmol CO$_2$ m$^{-2}$ s$^{-1}$
compared to 116.09 ± 15.3 µmol CO$_2$ m$^{-2}$ s$^{-1}$ (with $g_i$), and 239.65 ± 17.2 µmol CO$_2$ m$^{-2}$ s$^{-1}$ compared
to 108.18 ± 14.7 µmol CO$_2$ m$^{-2}$ s$^{-1}$ (without $g_i$).

Internal conductance to CO$_2$ ($g_i$) declined with water stress ($x^2=18.4, p < 0.01, n = 25$; Fig. 1e and
see Fig. 2b). In control trees however, $g_i$ remained consistent over the course of the growing season,
with no detectable differences between the different measurement periods. In control P. nigra trees,$g_i$ ranged between 1.75 ± 0.3 µmol m$^{-2}$ s$^{-1}$ Pa$^{-1}$ to 2.55 ± 0.8 µmol m$^{-2}$ s$^{-1}$ Pa$^{-1}$ (Fig. 1e). In droughted
trees, $g_i$ declined to 0.41 ± 0.01 µmol m$^{-2}$ s$^{-1}$ Pa$^{-1}$ to 0.60 ± 0.07 µmol m$^{-2}$ s$^{-1}$ Pa$^{-1}$ during the first and
second drought periods respectively. In trees recovering from water stress, $g_i$ was higher (3.38 ±
0.1 µmol m$^{-2}$ s$^{-1}$ Pa$^{-1}$), but this was within the measurement error of $g_i$ measured in control trees (2.55
± 0.8 µmol m$^{-2}$ s$^{-1}$ Pa$^{-1}$; Fig. 1e).

3.2. Model testing

All model configurations simulated observed rates of $A_{\text{net}}$ with high accuracy (Fig. 3a-c). Model 1
was seen to marginally over predict at low values of $A_{\text{net}}$ and under predict at high rates (Fig. 3a).
Both the intercept and slope of the regression line were found to be significantly different from zero
and one respectively (Table 4). Although the bias in the relationship was small, as shown by the 95%
confidence intervals (CIs) surrounding the slope and intercept. Further, the coefficient of
determination ($r^2$) was high (0.98), and the RMSD low (1.68 µmol CO$_2$ m$^{-2}$ s$^{-1}$; Table 4). Model 2
reduced the accuracy of simulated $A_{\text{net}}$ compared to Model 1 (Fig. 3b). The $r^2$ declined to 0.93 and the
RMSD increased to 5.86 µmol CO$_2$ m$^{-2}$ s$^{-1}$. Model 2 showed a pronounced bias to over-estimate $A_{\text{net}}$
that increased with increasing $A_{\text{net}}$. The scatter around the model regression line was much greater,
with larger CIs around the slope and intercept of the line (Table 4). No significant improvement in
modelled $A_{\text{net}}$ was seen with Model 3 compared to Model 1 (Fig. 3c). Although the $r^2$ were identical,
Model 3 generated a marginal increase in the RMSD (1.71 µmol CO₂ m⁻² s⁻¹) compared to Model 1. Model 3 showed a similar bias in predicted \( A_{\text{net}} \) to Model 1, over predicting at low values and under predicting at high values of \( A_{\text{net}} \) (Table 4). Model 3, however, improved the accuracy of simulated \( A_{\text{net}} \) compared to Model 2.

### 3.3. Model calibration

Model 1 maintained a good fit to the observed data, the \( r^2 \) remained reasonably high at 0.76 (Fig. 3d), and the RMSD was 5.34 µmol CO₂ m⁻² s⁻¹ (Table 4). The regression model line was not significantly different to the 1:1 line, however, the CIs surrounding the slope and intercept were substantially larger compared to the model testing stage. This suggests greater uncertainty in the fit between observed and modelled data. Model 2 generated a slight decrease in the accuracy of predicted \( A_{\text{net}} \) (Fig. 3e). Although the \( r^2 \) increased to 0.80, the RMSD also increased to 7.18 µmol CO₂ m⁻² s⁻¹, which is substantially larger than either Model 1 or Model 3. There was also significant bias in the model to over-estimate \( A_{\text{net}} \) which was less pronounced in Model 1 or Model 3 (Table 4; Fig. 3). The performance of Model 3 was almost identical to Model 1 (Fig. 3f). The \( r^2 \) was 0.76 and the RMSD only marginally increased to 5.35 µmol CO₂ m⁻² s⁻¹ (Table 4). Also, similar to Model 1, the regression line was not significantly different to the 1:1 line, and the CIs surrounding this line were similar. Compared to Model 2, Model 3 increased the accuracy of predicted \( A_{\text{net}} \). In each model configuration, the single parameter set clearly maintained a high level of accuracy of simulated \( A_{\text{net}} \), however there was noticeably more variation surrounding the model predicted values compared to the use of 'curve-specific' parameter values. This is to be expected, as each model was simulating observations from across the growing season with the use of a single calibrated parameter set, and it was seen that values inferred from each \( A_{\text{net}} - c_i \) and \( A_{\text{net}} - P_{\text{PFD}} \) curve varied across the growing season. Given the sensitivity of modelled \( A_{\text{net}} \) to \( V_{\text{max}} \) in particular, an exact match between the observed and modelled data could never be expected given the seasonal variation.

Comparison of Model 1 and Model 1_{def} (Table 4) shows the increased accuracy of using calibrated parameters as opposed to default model values. Use of the latter led to substantial under prediction of \( A_{\text{net}} \). The RMSD was increased from 5.34 µmol CO₂ m⁻² s⁻¹ to 9.42 µmol CO₂ m⁻² s⁻¹ (Table 4). These simulations using the default parameter values highlight the sensitivity of simulated \( A_{\text{net}} \) to the photosynthetic parameter \( V_{\text{max}} \). Values of \( a_{\text{int}} \), \( F_0 \) and \( D_c \) changed marginally between the default values and calibrated parameter sets. Additionally, in a previous Monte Carlo experiment to determine suitable values for \( D_c \) and \( F_0 \), it was seen that \( A_{\text{net}} \) displayed little sensitivity to these parameters. \( V_{\text{max}} \), however, changed significantly between simulations, with the calibrated values
being more than double the default value. Therefore, correct calibration of $V_{\text{max}}$ is key to improving predictions of leaf-level $A_{\text{net}}$.

3.4. Model validation

The accuracy of Model 1, the simplest model, and Model 3, the best performing 'alternative' model, were validated in this section.

3.4.1. Photosynthesis

The accuracy of simulated $A_{\text{net}}$ was improved by Model 1 compared to Model 3 (Fig. 3g & i). Model 1 is the JULES model in its original configuration and is the simplest model. The improvement in accuracy seen with Model 1 was slight, nevertheless, $r^2$ increased from 0.72 (Model 3) to 0.77 (Model 1). The RMSD decreased from 3.91 µmol CO$_2$ m$^{-2}$ s$^{-1}$ (Model 3) to 3.36 µmol CO$_2$ m$^{-2}$ s$^{-1}$ (Model 1; Table 4). Both models suggested bias in model predictions, both the regression line slope and intercept were different to the 1:1 line suggesting a tendency to over predict $A_{\text{net}}$ at higher values and under-predict at lower values (Table 4). Although this appears slightly less pronounced in Model 1, as the 95% CI surrounding the intercept in marginally smaller for Model 1.

3.4.2. Stomatal conductance

The accuracy of simulated $g_s$ was marginally improved in Model 3 (Fig. 3h & j). The $r^2$ was higher in Model 3 (0.86) than Model 1 (0.82), and the RMSD was lower in Model 3 (58.23 mmol H$_2$O m$^{-2}$ s$^{-1}$) than Model 1 (65.45 mmol H$_2$O m$^{-2}$ s$^{-1}$). In both models, the regression model slope was significantly different to one. The intercepts were not different to zero, however the CI surrounding the intercept was smaller in Model 3. Nevertheless, in both models, the accuracy of simulated $g_s$ was high.

4. Discussion

4.1. Photosynthetic parameters

In this work, estimates of $V_{\text{max}}$ made under the assumption of infinite $g_i$ were significantly lower than $V_{\text{max}}$ estimated assuming finite $g_i$. Therefore, in fast growing poplar genotypes, such as $P$. nigra used in this study, the assumption that internal CO$_2$ transfer is infinitely large as to have a negligible impact on the drawdown of CO$_2$ from $c_i$ to $c_e$ is invalid. Under well-watered conditions, values of


$V_{\text{max}}$ calculated from $A_{\text{net}} - c_c$ curves were, on average, 52% higher than values calculated from $A_{\text{net}} - c_i$ curves in P. nigra SRC trees. The differences between $c_i$ and $c_c$ based estimates of $V_{\text{max}}$ in this poplar genotype are large. There are no comparable studies of poplars in the literature, but Niinemets et al., (2009) reported $V_{\text{max}}$ calculated on a $c_c$ basis was 25% higher than on a $c_i$ basis in young fully mature leaves of field-grown olive trees. Bown et al., (2009) found mean values of $V_{\text{max}}$ calculated on a $c_c$ basis were 15.4% higher in pot grown Pinus radiata trees. Both these studies show smaller differences than in this study. Manter and Kerrigan (2004), however, reported differences in $c_i$ versus $c_c$ based estimates of $V_{\text{max}}$ in this study fall within the mid range of these values. Similar to these studies, this work highlights the impact $g_i$ has on estimates of this important photosynthetic parameter. In contrast, accounting for $g_i$ did not result in significantly higher estimates of $J_{\text{max}}$ in this study. Similarly, other studies have found that differences in $J_{\text{max}}$ calculated on a $c_i$ or $c_c$ basis are small (Flexas et al., 2007b; Niinemets et al., 2009; Warren, 2008).

In this study, values of $g_i$ for well-watered P. nigra trees ranged between 1.12 – 3.74 µmol m$^{-2}$ s$^{-1}$ Pa$^{-1}$, Flexas et al., (2008) report a range of $g_i$ from literature measured in Populus species of between 0.4 to 5.0 µmol m$^{-2}$ s$^{-1}$ Pa$^{-1}$, whilst this range is very large, it at least confirms that $g_i$ measured in this study falls well within this. The drought induced decline in $g_i$ was significant, indicating a substantial contribution of this diffusive limitation to photosynthetic carbon gain during the two periods of water stress. During drought, $g_i$ declined to 0.41 ± 0.01 µmol m$^{-2}$ s$^{-1}$ Pa$^{-1}$ and 0.60 ± 0.07 µmol m$^{-2}$ s$^{-1}$ Pa$^{-1}$ in P. nigra trees during the first and second drought period respectively. Although values of $g_i$ reported in this study are relatively high, there is noticeable variation in the measurements. Consequently, at times it is possible $g_i$ may be limiting to photosynthesis even under well-watered conditions. This identifies a potential target for breeding programmes to improve yields and water-use efficiency (Centritto et al., 2009).

Unlike the work of Galmés et al., (2007), this study found that values of $V_{\text{max}}$ and $J_{\text{max}}$ in re-watered trees following the first drought period were almost two-fold higher than those of control trees. Poplar species are typically pioneers of riparian ecosystems, as such, many poplar species are notoriously susceptible to, and display limited sensitivity to drought (Hall and Allen, 1997; Monclus et al., 2006). In this study, $A_{\text{net}}$ and $g_s$ in P. nigra trees only declined once the soil moisture deficit was below an apparent critical threshold of soil moisture content (11-18 % vol.). This threshold response has been observed in other genotypes of poplar (Hall and Allen, 1997). In addition to reduced $A_{\text{net}}$ and $g_s$, the leaves of P. nigra trees in this study were observed to yellow, this was followed by leaf shedding. This is a common mechanism of acclimation to drought conditions in fast growing species, and before leaf senescence, nitrogen is assimilated out of leaves (Lambers et al.,
There is a strong correlation between leaf nitrogen content and photosynthetic capacity since the proteins of the thylakoids and Calvin cycle (e.g. Rubisco, the enzyme involved in CO₂ fixation) represent the majority of leaf nitrogen (Evans, 1989). Consequently, depletion of leaf nitrogen as a result of drought leads to photosynthetic down-regulation resulting from necessary adjustments to the biochemical photosynthetic capacity. During drought, Rubisco content and/or activity has been observed to decline as a result of either reduced leaf nitrogen content and/or different partitioning of leaf nitrogen among photosynthetic enzymes (Bota et al., 2004; Castrillo et al., 2001; Grassi and Magnani, 2005; Parry et al., 2002; Tezera et al., 2002) leading to a decline in $V_{\text{max}}$ and $J_{\text{max}}$. Large increases in biochemical photosynthetic capacity upon re-watering may result from the allocation of assimilated nitrogen back to the leaves, or partitioning of more nitrogen to photosynthetic enzymes such as Rubisco, to maximise growth once favourable environmental conditions return. This strategy may be symptomatic of the life strategy of this poplar species as a fast-growing pioneer.

The observed decline in $V_{\text{max}}$ and $J_{\text{max}}$ during drought was apparent when measured at both $c_c$ and $c_i$, suggesting there is a biochemical limitation to photosynthetic capacity during periods of water stress. Additionally, the up-regulation of these parameters upon re-watering suggests that $V_{\text{max}}$ and $J_{\text{max}}$ are highly plastic. This has implications for land-surface modelling and the representation of drought within these models. Currently in the JULES $A_{\text{net}}$ - $g_s$ model, water stress effects on $A_{\text{net}}$ and $g_s$ are accounted for by applying a normalised soil moisture dependent function to $A_{\text{net}}$ directly (Best et al., 2011). The potential (i.e. non water stressed) rate of $A_{\text{net}}$ is calculated and then modified by the soil moisture stress function. The rate of $g_s$ in response to water stress is then modified accordingly, and is derived from a semi-empirical function that relates $g_s$ to modelled $A_{\text{net}}$ and intercellular/atmospheric CO₂ concentration (Best et al., 2011). However, the results of this work suggest it may be more appropriate to apply the soil moisture stress function directly to one or more parameters in the photosynthesis model itself, such as $V_{\text{max}}$ and $J_{\text{max}}$, to better represent this biochemical limitation to photosynthetic capacity during drought.

The measured reduction in $V_{\text{max}}$ and $J_{\text{max}}$ clearly shows there was a biochemical limitation to photosynthesis during drought in *P. nigra* trees. However, the threshold function that described the decline in $g_s$ with increasing water stress in *P. nigra* trees in this study was mirrored by $A_{\text{net}}$, which clearly suggests a stomatal limitation to photosynthetic carbon gain. Further, the decline in $g_i$ with drought suggests an additional diffusional limitation. The consensus in the literature on the main processes governing photosynthetic limitation during drought is diffusional processes at mild to moderate drought, and biochemical processes during severe water stress (Flexas et al., 2006; Flexas et al., 2004a; Flexas et al., 2008; Grassi and Magnani, 2005; Lawlor and Cornic, 2002). The current representation of water stress in the JULES $A_{\text{net}}$ - $g_s$ model uses a linear function to model the
response of $A_{\text{net}}$ and $g_s$ to drought, which is evidently not appropriate for all species. Moreover, the results of this work and the literature suggest that future work should consider modelling drought through biochemical and diffusional controls, since it is the sum of both these processes that determine water stressed $A_{\text{net}}$. Initial work by Egea et al., (2011) in this area demonstrated that in the coupled $A_{\text{net}} - g_s$ model used in their work, it was necessary to combine both diffusional and biochemical limitations of $A_{\text{net}}$ to accurately capture observed functional relationships between $A_{\text{net}}$ and $g_s$ in response to drought.

For the purposes of this work, $V_{\text{max}}$ was parameterised directly using field measurements of $V_{\text{max}}$ from $P. \text{nigra}$ trees. Currently, in the JULES model, $V_{\text{max}}$ can be parameterised with a distribution of leaf nitrogen (N) that allows $V_{\text{max}}$ to decrease from top to bottom of the canopy (see Eq. (28) from Clark et al., 2011). However, currently, leaf N, and hence $V_{\text{max}}$, does not vary temporally. This means that the high plasticity observed in $V_{\text{max}}$ in this study, when stressed trees were re-watered, would be difficult to represent in the model. In addition, other studies have shown that $V_{\text{max}}$ decreases over the course of the growing season (Grassi et al., 2005; Niinemets et al., 1999; Wilson et al., 2001), which again is not possible to simulate without temporal variation in $V_{\text{max}}$ or leaf N concentration. It has been shown that modelled seasonal trends in carbon fluxes are explained best with a temporally varying $V_{\text{max}}$ (Wang et al., 2004; Wilson et al., 2001). Therefore, a time-varying $V_{\text{max}}$ would allow for greater plasticity in this parameter as is observed in the field. It may be possible to link water stress effects on plant $A_{\text{net}}$ and $g_s$ to changes in leaf N concentration, although this would likely require a prognostic model of nitrogen availability and uptake by plants. Nevertheless, results from this work and the literature suggest that the photosynthetic capacity of plants changes with time and environmental conditions, which indicates there should be greater flexibility in the temporal parameterisation of $V_{\text{max}}$. This may be possible to do with respect to the different plant functional types (PFTs) used in land-surface models, however, comparing the large increase in $V_{\text{max}}$ upon re-watering observed in this study with the results of Galmés et al., (2007) shows that there is significant variation between species in the way this parameter responds to water stress and its alleviation.

### 4.2. Does inclusion of $g_i$ in a coupled $A_{\text{net}} - g_s$ model improve the accuracy of simulated $A_{\text{net}}$ and $g_s$?

The results of model testing, calibration and validation from this work suggest there is no significant improvement in the accuracy of modelled $A_{\text{net}}$ from the inclusion of $g_i$ to necessitate the addition of this process to the JULES $A_{\text{net}} - g_s$ model parameterisation (Table 4; Fig. 3). Testing, calibration and validation of the three model configurations agreed and showed that the performance of Model 1 (no
$g_i$ and Model 3 (with $g_i$), were very similar. Both model configurations shared high $r^2$ and low RMSD (Table 4). In this work, $g_i$ was included in model configurations 2 and 3 as a constant, using the mean $g_i$ value measured in unstressed *P. nigra* trees over the course of the experimental period. Simulated $A_{net}$ and $g_s$ values in Model 1 used an ‘apparent’ value of $V_{max}$ (i.e. determined at $c_i$), whereas Model 2 and 3 used a ‘true’ value of $V_{max}$ (i.e. determined at $c_c$). The true $V_{max}$ value is estimated by explicitly modelling the extra diffusional pathway from $c_i$ to $c_c$, whereas the apparent value inherently includes this information about $g_i$. Therefore, including $g_i$ in the model configurations did not have a large impact on the accuracy of modelled $A_{net}$ and $g_s$ in the coupled model since the value of $V_{max}$ used in each model configuration compensated for the presence/absence of this additional pathway.

$V_{max}$ is generally regarded as a good indicator of photosynthetic capacity and is considered a directly transferable parameter to calibrate models of ecosystem carbon exchange. However, given the difference between estimates of $V_{max}$ determined at either $c_c$ or $c_i$, the assumed transferability of this parameter from measurements to models and between models is not so straightforward. It must be ensured that values of $V_{max}$ used in carbon exchange models are correct for the assumptions underlying the model (i.e. determination of photosynthesis on a $c_i$ or $c_c$ basis). If, for example, a true $V_{max}$ value were used to parameterise a photosynthesis model that calculated $A_{net}$ at $c_i$, it is likely that $A_{net}$ would be grossly over-estimated because of the significantly higher value of $V_{max}$, and the lack of explicit representation of the $c_i$ to $c_c$ CO$_2$ transfer. This would likely propagate into errors in the prediction of ecosystem NPP at the larger scale.

Simulated $A_{net}$ using a default model set of parameter values highlighted the sensitivity of simulated $A_{net}$ to $V_{max}$. In this simulation, the accuracy of predicted $A_{net}$ was greatly reduced; the RMSD was almost two times higher the RMSD from model configurations 1 to 3 which used a calibrated value of $V_{max}$ for *P. nigra* (Table 4). This high sensitivity to $V_{max}$ again emphasises the importance of correctly matching values of $V_{max}$ (i.e. true or apparent) to the correct assumptions of the photosynthesis model. Moreover, however, it suggests that the uncertainty surrounding values of $V_{max}$ used to parameterise land-surface models potentially has a much greater impact on the simulation of $A_{net}$ than other fine-scale leaf-level processes such as internal CO$_2$ transfer. Recent work by Bonan et al., (2011) on improving canopy processes in the Community Land Model version 4 (CLM4) concludes that uncertainty in the parameter $V_{max}$ produces effects of comparable magnitude as model structural errors, and that currently, $V_{max}$ remains a poorly constrained, model-dependent parameter.
Represented as a constant, unstressed, value of $g_i$ in different configurations of the coupled $A_{net} - g_s$ model, the inclusion of $g_i$ to simulate the extra diffusional pathway of CO$_2$ from $c_i$ to $c_c$ during photosynthesis did not improve the accuracy of simulated $A_{net}$ and $g_s$. Given the current limited understanding of $g_i$, it was necessary to take such an approach. Few models simulating whole plant carbon exchange consider the internal transfer of CO$_2$ as part of their parameterisation. The ISBA-Ags land-surface model (Calvet et al., 1998), C-TESSEL surface exchange scheme (Voogt et al., 2005), and the SPA model (Williams et al., 1996) are the few examples of models that do include $g_i$. In these models, $g_i$ is also parameterised as a constant value. Similar to $g_s$, however, $g_i$ is known to respond to changing environmental conditions in the long (days/weeks) and short (minutes) term (Flexas et al., 2008; Warren, 2008). A process-based implementation of $g_i$ may therefore be more desirable, currently, however, there are significant research gaps that would make this difficult. These include the variation in $g_i$ with temperature, the response of $g_i$ to light and vapour pressure deficit, and scaling $g_i$ within the canopy (Bernacchi et al., 2002; Flexas et al., 2008; Niinemets et al., 2006; Warren, 2008; Warren and Adams, 2006; Warren and Dreyer, 2006). Consequently, greater understanding of the interaction of $g_i$ with its environment is needed before a truly process-based approach can be used to model this additional CO$_2$ pathway. Nevertheless, this work contributes to our understanding of the impact of $g_i$ in land-surface modelling, and raises further issues that may be of equal importance to address before consideration of additional fine-scale leaf-level processes such as $g_i$. These include the uncertainty surrounding values of $V_{max}$ used in land-surface models, and the representation of water stress effects on vegetation within such models.

Acknowledgements:

We would like to thank Lina Mercado, Martin De Kauwe and two anonymous reviewers for reading and improving drafts of this manuscript. We would also like to acknowledge the time and help from Lina Mercado given during the analysis of this work. We would like to thank Mike Morecroft for advice on the experimental design, and John Krebs field station, Oxfordshire for supporting our field work. We would like to thank Mike Cotton at Southampton University, John Fuller, Richard Hailstone and Julie Hailstone for their assistance preparing and setting up the experimental plant material. RJO was supported by a research studentship from the Natural Environment Research Council (no. NER/S/A/2006/14314).
References


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<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Temperature dependencies</td>
<td>$Q_{10}$ function</td>
<td>Exponential function</td>
<td>Exponential function</td>
</tr>
<tr>
<td></td>
<td>(see Collatz et al., 1991)</td>
<td>(see Sharkey et al., 2007)</td>
<td>(see Sharkey et al., 2007)</td>
</tr>
<tr>
<td>Rubisco kinetic constants</td>
<td>$Q_{10}$ temp.</td>
<td>Values determined in vivo at $c_{i}$ (see Sharkey et al., 2007)</td>
<td>Values determined in vivo at $c_{i}$ (see Sharkey et al., 2007)</td>
</tr>
<tr>
<td></td>
<td>Coefficients</td>
<td>(see Collatz et al., 1991)</td>
<td></td>
</tr>
</tbody>
</table>

**Table 1.** Differences between the three model configurations: $V_{\text{max}}$ (µmol CO$_2$ m$^{-2}$ s$^{-1}$), maximum carboxylation rate of Rubisco; $\alpha_{\text{int/app}}$ (mol CO$_2$ mol$^{-1}$ PAR; mol e$^{-}$ mol$^{-1}$ photons respectively), intrinsic/apparent quantum efficiency; $g_{i}$ (µmol CO$_2$ m$^{-2}$ s$^{-1}$ Pa$^{-1}$), the internal conductance to CO$_2$; $J_{\text{max}}$ (µmol e$^{-}$ m$^{-2}$ s$^{-1}$), maximum rate of electron transport; $F_{0}$, $c_{i}/c_{a}$ ratio for specific humidity deficit in canopy; $D_{c}$ (kg kg$^{-1}$), the critical humidity deficit.

<table>
<thead>
<tr>
<th>Exponential function</th>
<th>$Q_{10}$ function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value at 25°C</td>
<td>c</td>
</tr>
<tr>
<td>$K_{c}$ (Pa)</td>
<td>16582</td>
</tr>
<tr>
<td>$K_{r}$ (Pa)</td>
<td>27.238</td>
</tr>
<tr>
<td>$\Gamma^*$ (Pa)</td>
<td>3.743</td>
</tr>
<tr>
<td>Used for normalising</td>
<td></td>
</tr>
<tr>
<td>$R_{d}$ (µmol CO$_2$ m$^{-2}$ s$^{-1}$)</td>
<td>18.7145</td>
</tr>
<tr>
<td>$V_{\text{max}}$ (µmol CO$_2$ m$^{-2}$ s$^{-1}$)</td>
<td>26.355</td>
</tr>
<tr>
<td>$J_{\text{max}}$ (µmol e$^{-}$ m$^{-2}$ s$^{-1}$)</td>
<td>17.71</td>
</tr>
<tr>
<td>$g_{i}$ (µmol CO$_2$ m$^{-2}$ s$^{-1}$ Pa$^{-1}$)</td>
<td>20.01</td>
</tr>
</tbody>
</table>

**Table 2.** Exponential and $Q_{10}$ temperature response functions for photosynthetic parameters and Rubisco enzyme kinetic parameters (plus values at 25°C for model fitting). Values are taken from Bernacchi et al., (2002; 2001) and Bernacchi et al., (2003) for the exponential functions. Values are from Collatz et al., (1991) and Niinemets et al., (2009) for the $Q_{10}$ response functions: $c$, scaling constant; $\Delta H_{a}$, enthalpy of activation; $\Delta H_{d}$, enthalpy of deactivation; $\Delta S$, entropy; $K_{c}$, Michaelis-Menten constant of Rubisco for O$_2$; $K_{r}$, Michaelis-Menten constant of Rubisco for CO$_2$; $\Gamma^*$, chloroplastic CO$_2$ photocompensation point in the absence of mitochondrial respiration; $R_{d}$, dark respiration; $V_{\text{max}}$, maximum carboxylation rate of Rubisco; $J_{\text{max}}$, maximum rate of electron transport; $g_{i}$, the internal conductance to CO$_2$. This table is partly reproduced from Sharkey et al., (2007).
<table>
<thead>
<tr>
<th>Without $g_i$</th>
<th>With $g_i$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta$</td>
<td>$\Delta$</td>
</tr>
<tr>
<td>$V_{\text{max}}$</td>
<td>$V_{\text{max}}$</td>
</tr>
<tr>
<td>27.92</td>
<td>59.11</td>
</tr>
<tr>
<td>100.73</td>
<td>120.69</td>
</tr>
<tr>
<td>96.91</td>
<td>38.41</td>
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<tr>
<td>110.00</td>
<td>186.54</td>
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<tr>
<td>81.93</td>
<td>114.39</td>
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<tr>
<td>63.71</td>
<td>97.62</td>
</tr>
<tr>
<td>64.26</td>
<td>107.24</td>
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<tr>
<td>82.43</td>
<td>126.00</td>
</tr>
<tr>
<td>42.70</td>
<td>51.04</td>
</tr>
<tr>
<td>50.41</td>
<td>92.80</td>
</tr>
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<td>47.88</td>
<td>80.50</td>
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<td>67.34</td>
<td>109.41</td>
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<td>77.07</td>
<td>124.77</td>
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<td>60.59</td>
<td>63.45</td>
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<tr>
<td>70.48</td>
<td>109.75</td>
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<tr>
<td>73.05</td>
<td>109.75</td>
</tr>
<tr>
<td><strong>69.84</strong></td>
<td><strong>99.47</strong></td>
</tr>
<tr>
<td>± 5.47</td>
<td>± 8.99</td>
</tr>
</tbody>
</table>

Table 3. Parameter values from each individual $A_{\text{net}} - c_i$ and $A_{\text{net}} - P_{\text{FD}}$ response curve measured for $P. \text{nigra}$. Values in bold show the mean ± S.E. The symbols indicate which model the parameter values were used in: ♦ Model 1, Δ Model 2, † Model 3. $V_{\text{max}}$ (µmol CO$_2$ m$^{-2}$ s$^{-1}$), maximum carboxylation rate of Rubisco estimated with and without $g_i$; $J_{\text{max}}$ (µmol e$^{-}$ m$^{-2}$ s$^{-1}$), maximum rate of electron transport; $g_i$ (µmol CO$_2$ m$^{-2}$ s$^{-1}$ Pa$^{-1}$), the internal conductance to CO$_2$; $a_{\text{int/app}}$ (mol CO$_2$ mol$^{-1}$ quanta; mol e$^{-}$ mol$^{-1}$ quanta respectively), intrinsic/apparent quantum efficiency; $F_0$, $c_i/c_a$ ratio for specific humidity deficit in canopy; $D_c$, (kg kg$^{-1}$), the critical humidity deficit.
<table>
<thead>
<tr>
<th>Regression fit</th>
<th>$r^2$</th>
<th>95% CI Intercept</th>
<th>95% CI Slope</th>
<th>Correlation $F$ (1, 190 d.f)</th>
<th>$P$</th>
<th>RMSD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Model Testing</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>$y = -1.26 - 1.06x$</td>
<td>0.98</td>
<td>0.38 ***</td>
<td>0.02 ***</td>
<td>9412</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Model 2</td>
<td>$y = 0.14 - 0.77x$</td>
<td>0.93</td>
<td>0.69 ***</td>
<td>0.03 ***</td>
<td>2413</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Model 3</td>
<td>$y = -0.97 - 1.08x$</td>
<td>0.98</td>
<td>0.37 ***</td>
<td>0.02 ***</td>
<td>9678</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>Model Calibration</strong></td>
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<td></td>
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</tr>
<tr>
<td>Model 1</td>
<td>$y = -1.16 - 1.00x$</td>
<td>0.76</td>
<td>1.41</td>
<td>0.08</td>
<td>614.3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Model 2</td>
<td>$y = 1.08 - 0.72x$</td>
<td>0.80</td>
<td>1.13 *</td>
<td>0.09 ***</td>
<td>775</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Model 3</td>
<td>$y = -1.44 - 1.09x$</td>
<td>0.76</td>
<td>1.45</td>
<td>0.09</td>
<td>594.9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Model _def</td>
<td>$y = 1.12 - 1.71x$</td>
<td>0.71</td>
<td>1.43</td>
<td>0.16 ***</td>
<td>463.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>Model Validation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>$A_{net}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>$y = 1.45 - 0.84x$</td>
<td>0.77</td>
<td>0.72 ***</td>
<td>0.06 ***</td>
<td>763.4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Model 3</td>
<td>$y = 1.96 - 0.77x$</td>
<td>0.72</td>
<td>0.78 ***</td>
<td>0.06 ***</td>
<td>589.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>$g_s$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>$y = 8.50 - 0.90x$</td>
<td>0.82</td>
<td>13.61</td>
<td>0.05 ***</td>
<td>1083</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Model 3</td>
<td>$y = 1.89 - 0.93x$</td>
<td>0.86</td>
<td>12.32</td>
<td>0.05 ***</td>
<td>1403</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Table 4. Quantitative measures of the ability of the models to predict observed rates of $A_{net}$ during model testing and model parameterisation. The units of RMSD are ($\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$) $A_{net}$ and (mmol H$_2$O m$^{-2}$ s$^{-1}$) for $g_s$. CI refers to the 95% confidence interval surrounding the estimate of the regression line intercept/slope. The stars indicate whether the intercept/slope is significantly different to zero/one respectively: *** p < 0.001, ** p < 0.01, * p < 0.05. $F^*$ indicates *P. nigra* is analysed on 1, 230 d.f. for validation.
Fig. 1. Values of a) the maximum carboxylation velocity ($V_{max}$) estimated with internal conductance to CO$_2$ ($g_i$), b) $V_{max}$ estimated without $g_i$, c) the maximum rate of electron transport ($J_{max}$) estimated with $g_i$, d) $J_{max}$ estimated without $g_i$, and e) $g_i$. For each measurement period, the mean is shown ± the standard error. ND and NW are stressed and control *P. nigra* trees respectively. Measurement periods over the course of the experiment are denoted by; Predrt.1 = pre-drought 1 (first drought); Drt.1 = first drought; Rec = recovery (re-watering of stressed trees); Predrt.2 = pre-drought 2 (second drought); Drt.2 = second drought.
Fig. 2. a) Daily maximum (solid line) and minimum (dotted line) air temperatures (°C), and b) hourly soil moisture content (% vol.) over the experimental period (2nd June – 1st October 2008). Grey arrows indicate the periods of imposed drought. NW, well-watered (control) *P. nigra* trees; ND, drought treated *P. nigra* trees.
Fig. 3. Model testing, observed vs. predicted $A_{net}$ ($\mu$mol m$^{-2}$ s$^{-1}$) and $g_s$ (mmol m$^{-2}$ s$^{-1}$) shown for *P. nigra*: a, b & c) model testing, d, e & f) model calibration, g, h, I & j) model validation. The regression line (dotted line) and $r^2$ are shown, along with the 1:1 line (solid line).