2   **Reconciling the optimal and empirical approaches to modelling**

3   **stomatal conductance**

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24   **Running head: Reconciling optimal and empirical stomatal models**

25 **Abstract**

26 Models of vegetation function are widely used to predict the effects of climate change on

27 carbon, water and nutrient cycles of terrestrial ecosystems, and their feedbacks to climate.

28 Stomatal conductance, the process that governs plant water use and carbon uptake, is

29 fundamental to such models.

30 In this paper, we reconcile two long-standing theories of stomatal conductance. The

31 empirical approach, which is most commonly used in vegetation models, is

32 phenomenological, based on experimental observations of stomatal behaviour in response

33 to environmental conditions. The optimal approach is based on the theoretical argument

34 that stomata should act to minimise the amount of water used per unit carbon gained.

35 We reconcile these two approaches by showing that the theory of optimal stomatal

36 conductance can be used to derive a model of stomatal conductance that is closely

37 analogous to the empirical models. Consequently we obtain a unified stomatal model

38 which has the same form as existing empirical models, but which now provides a

39 theoretical interpretation for model parameter values.

40 The slope parameter of the model is predicted to increase with growth temperature and

41 with the marginal water cost of carbon gain. The new model is fitted to a range of

42 datasets ranging from tropical to boreal trees. The slope parameter is shown to vary with

43 growth temperature, as predicted, and also with plant functional type. The model is

44 shown to correctly capture responses of stomatal conductance to changing atmospheric

45 CO_2, and thus can be used to test for stomatal acclimation to elevated CO_2. The

46 reconciliation of the optimal and empirical approaches to modelling stomatal

47 conductance provides a useful theoretical framework for analyzing and simulating

48 stomatal behaviour.

49   Keywords: stomatal conductance, coupled conductance and photosynthesis models,

50 stomatal optimisation, marginal water cost of carbon

51

Introduction

Models of vegetation function have a major role to play in advancing our understanding of terrestrial ecosystem responses to global change. Land surface schemes are integral to climate models (Sellers *et al*. 1997, Pitman 2003), while dynamic vegetation models are used to predict climate impacts on biospheric carbon, nutrient and water cycles (e.g. Scholze *et al*. 2006, Piao *et al*. 2007, Sitch *et al*. 2008, Ostle *et al*. 2009). Fundamental to all these vegetation function models are descriptions of the key processes of plant carbon uptake (photosynthesis) and water use (transpiration).

Photosynthesis is widely represented using a mechanistic model in which rates of key processes are related to environmental drivers including the concentration of atmospheric CO_2, light and temperature (Farquhar *et al*. 1980). This mechanistic model has acted as a framework for considerable ecophysiological research, with the result that we now have a good understanding of how photosynthetic rates vary among species and ecosystems (e.g. Wullschleger 1993, Kattge *et al*. 2009), and how photosynthesis acclimates to changes in temperature and atmospheric CO_2 (e.g. Medlyn *et al*. 1999, Medlyn *et al*. 2002, Ellsworth at al. 2004, Ainsworth and Rogers 2007, Kattge and Knorr 2007).

In contrast to the mechanistic model of photosynthesis, transpiration is generally modeled using an empirical representation of stomatal conductance. Experiments have shown that stomatal conductance (g_s) is typically correlated with photosynthesis (A) (Wong *et al*. 1979), but that the ratio of g_s : A varies with atmospheric humidity (Ball *et al*. 1987). These observations have been used to develop simple, empirical models of g_s (Ball *et al*. 1987, Leuning 1995). The use of these models is widespread because parameters are readily estimated from data and the models are simple enough to implement at global scales.

However, because these stomatal conductance models are empirical, their parameters have no meaning attached. Consequently, there is little understanding of how the parameters vary with species or acclimate to changes in climate, and many models simply assume that the parameters are constant for all C_3 species (e.g. Sitch *et al*. 2003, Krinner

81 *et al.* 2005, Law *et al.* 2006). A successful theoretical model of stomatal behaviour is a

82 high priority for vegetation modelers because it would provide a framework for research

83 into acclimation and adaptation of stomatal control of water and C fluxes.

84 There is a long-standing theory of optimal stomatal behaviour (Cowan & Farquhar 1977).

85 This theory is based on the idea that stomata should act to maximize carbon gain

86 (photosynthesis, A) while minimizing water loss (transpiration, E). That is, the optimal

87 stomatal behaviour would be to minimize the integrated sum of

88 $\qquad$ E - λ A $\hfill$ (1)

89 where λ (mol H_2O mol^{-1} C) is a parameter representing the marginal water cost of plant

90 carbon gain. This general theory is widely accepted (e.g. Bonan 2008 p244) but it is

91 rarely used in models. Although model implementations of this theory have been

92 attempted (e.g. Hari *et al.* 1986, Lloyd 1991, Arneth *et al.* 2002, Katul *et al.* 2009a,

93 among others), several issues have restricted wider use of these implementations. A key

94 issue has been parameterisation – values of λ have been perceived as difficult to

95 estimate, and questions have been raised as to the timescale on which λ might remain

96 constant (Cowan and Farquhar 1977, Thomas et al. 1999). Also, previous

97 implementations do not correctly capture stomatal responses to atmospheric CO_2

98 concentration.

99 In this paper, we reconcile the optimal and empirical models of stomatal conductance.

100 We demonstrate that, under reasonable and generally applicable simplifying

101 assumptions, the optimal stomatal conductance model is, in fact, functionally equivalent

102 to the widely-used empirical stomatal model. We derive a unified model that has the form

103 of the empirical stomatal models but that is based on the optimal stomatal conductance

104 theory. The benefit of this unified model is that it gives a biological interpretation for

105 model parameters that previously were regarded as empirical constants. We demonstrate

106 that the key model parameter (the slope parameter) varies significantly among species,

107 and discuss hypotheses for this variation. This analysis provides a useful quantitative

108   framework for research into the long-term acclimation and adaptation of stomatal

109   function to environmental conditions.

110

111   **Background**

112   *Empirical models*

113   The model of Ball *et al*. (1987) is based on the observation that stomatal conductance is

114   strongly correlated with assimilation rate (Wong *et al*. 1979). Based on a series of leaf

115   gas exchange experiments, Ball *et al*. (1987) developed the following empirical

116   expression for g_s:

117            $$g_s = g_0 + g_1\,(A\,h_r\,/\,C_a)$$ (2)

118   where g_0 and g_1 are fitted parameters, *A* is net assimilation rate (μmol m^{-2} s^{-1}), h_r is

119   relative humidity at the leaf surface (dimensionless), and C_a is atmospheric CO_2

120   concentration at the leaf surface (μmol mol^{-1}). This model has been criticized because it

121   can be shown that stomata sense transpiration and/or peristomatal water fluxes, rather

122   than relative humidity (Aphalo and Jarvis 1991, Mott and Parkhurst 1991; Eamus *et al*.

123   2008). An alternative model incorporating an empirical dependence on leaf-to-air vapour

124   pressure deficit (*D*, kPa), a proxy for transpiration, was developed by Leuning (1995).

125   Leuning (1995) considered two alternative forms for the dependence on D, a linear and

126   hyperbolic dependence, and found that a hyperbolic dependence provided a better fit to

127   experimental data. The resulting model has the following form:

128            $$g_s = g_0 + g_1 \frac{A}{(C_a - \Gamma)(1 + D/D_0)}$$ (3)

129   where Γ is the CO_2 compensation point of assimilation in the presence of dark

130   respiration. This model has three empirically fitted parameters, g_0, g_1 and D_0.

131   These models (eqns 2 and 3) are widely used because they are straightforward to

132   parameterise from leaf-scale data, are easy to implement at large scales, and nonetheless

133   appear to capture the fundamentals of stomatal behaviour. However, there are several

134   important criticisms that can be made of both models. As noted already, equation (2) is

135   incorrect in its assumption of a dependence on h_r. A significant practical problem with

136   equation (3) is that the parameters g_1 and D_0 are very strongly correlated. This correlation

137   means that the parameters are difficult to estimate from data with confidence, and

138   differences in the parameters among datasets cannot be clearly interpreted (e.g. Medlyn *et*

139   *al*. 2005). A model with formally identifiable parameters (i.e. one in which parameters

140   are not correlated) is desirable.

141   The major criticism of both models, however, is that they are empirical in nature. They

142   have been developed from experimental observations, rather than from any mechanistic

143   understanding or theory of stomatal behaviour. This empirical basis is unsatisfactory

144   because it means that we lack confidence in applying the model in novel situations (such

145   as under increasing atmospheric CO_2 concentration). It also means that we have no

146   theoretical basis for predicting or interpreting differences in parameter values among

147   species and vegetation types. Lacking this basis, the parameters are simply assumed

148   constant for all C_3 vegetation in many regional and global models (e.g. Krinner *et al*.

149   2005), while in other models, parameter values are tuned to match large-scale

150   observations (e.g. Cox 2001, Oleson *et al*. 2004).

151   *Optimal stomatal conductance model*

152   A theory of optimal stomatal behaviour was developed by Cowan & Farquhar (1977).

153   This theory postulates that stomata should act to maximize carbon gain (photosynthesis,

154   *A*) whilst at the same time minimizing water lost (E, transpiration). That is, the optimal

155   stomatal conductance is obtained when the following expression is minimized:

156          $$\int_{t_1}^{t_2} (E(t) - \lambda A(t))\, dt \qquad\qquad (4)$$

157   where λ (mol H_2O mol^{-1} C) is a parameter describing the marginal water cost of carbon

158   gain. Cowan & Farquhar (1977) showed, using calculus of variations, that minimizing

159   this expression leads to the following optimization constraint:

160
$$\frac{\partial E}{\partial A} = \lambda \qquad (5)$$

161   Hari *et al*. (1986) combined this constraint with a very simple photosynthetic model in

162   which *A* was assumed proportional to intercellular CO_2 concentration, C_i, and a function

163   of incident light, f(I), i.e.

164
$$A = C_i \, f(I) \qquad (6)$$

165   They obtained the following expression for optimal stomatal conductance, g_s^*:

166
$$g_s^* = f(I)\sqrt{\frac{C_a \lambda}{1.6 D} - 1} \qquad (7)$$

167   where D is vapour pressure deficit. We note that Hari *et al*. (1986) defined their

168   parameter λ as the inverse of that used by Cowan & Farquhar (1977), whereas here we

169   use Cowan's definition of the parameter and so have modified Hari *et al*.'s expression

170   accordingly. This model was found to give a very good fit to continuous measurements of

171   transpiration and photosynthesis in a boreal Scots pine forest (Hari *et al*. 1999, Mäkelä *et*

172   *al*. 2004, Kolari *et al*. 2007), and has been implemented in a canopy photosynthesis

173   model (Mäkelä *et al.* 2006). Katul *et al.* (2009a) explored the properties of this model and

174   found that it was consistent with observed responses of g_s, *E*, and the ratio $C_i : C_a$ to D.

175   However, this model does not correctly capture the response of g_s to changes in

176   atmospheric CO_2, because it predicts that g_s should increase with increasing C_a. This

177   problem arises because of the simplifying assumption that *A* is proportional to C_i (eqn 6).

178   Arneth *et al*. (2002) combined the relationship with the more realistic model of

179   photosynthesis developed by Farquhar *et al*. (1980). This model assumes that the

180   photosynthetic rate is limited by either RuBP regeneration, in which case

181
$$A = \frac{J}{4} \frac{C_i - \Gamma^*}{C_i + 2\Gamma^*} - R_d \qquad (8)$$

182   where J is the rate of electron transport, Γ^* is the CO_2 compensation point in the absence

183   of dark respiration, and R_d is the dark respiration rate; or it is limited by the rate of

184   carboxylation, in which case

185
$$A = V_{c\max} \frac{C_i - \Gamma^*}{C_i + K_m} - R_d \qquad (9)$$

186   where V_{cmax} is the maximum rate of Rubisco activity and K_m is the Michaelis-Menten

187   coefficient for Rubisco kinetics. Note that mesophyll conductance (Niinemets *et al*. 2009)

188   is implicit in this formulation of the model and is not considered explicitly here. Arneth *et*

189   *al*. (2002) showed that, when photosynthesis is represented in this way, the optimal C_i

190   depends on λ according to two quadratic equations corresponding to the two different

191   limitations (see Appendix). In this paper, we focus only on the first limitation to

192   photosynthesis (eqn 8); the reasons for this choice are fully explained in the Discussion.

193

194   **Theory**

195   We coupled the optimal stomatal control model with equation (8), using the quadratic

196   equation obtained by Arneth *et al*. (2002) as a starting point. As described in the

197   Appendix, we then derived the following approximation for the optimal stomatal

198   conductance:

199
$$g_s^* = g_0 + g_1 \frac{A}{C_a \sqrt{D}} \qquad (10)$$

200
201   The analytical expression in equation (10) is closely analogous to the empirical models

202   described by equations (2) and (3). We term equation (10) the unified stomatal model,

203   because it has the same form as the empirical models but is derived from the optimal

204   model, thus combining both approaches into the one model.

205

206   The parameter g_1 can be directly obtained by fitting to data in the same way as is usually

207   done with the empirical models. However, we now have a theoretical interpretation for

208   the slope parameter g_1: as shown in the Appendix, g_1 increases linearly with the

209   combination of terms:

210   $$g_1 \sim \sqrt{\Gamma^* \lambda} \qquad (11)$$

211

212   That is, the slope parameter should increase with the marginal water cost of carbon λ, and

213   with the CO_2 compensation point Γ^*.

214

215   We compared the exact solution of the coupled stomatal – photosynthesis model with the

216   simplified model given by equation (10), and found it is an excellent approximation to the

217   optimal stomatal conductance over a realistic range of values of light, D, C_a, and λ

218   (Figure 1a). The mean absolute deviation between the simplified model and the numerical

219   solution was 0.0044 mol m^{-2} s^{-1}. Figure 1b demonstrates the close one-to-one relationship

220   between the slope parameter g_1 and the parameter combination $\sqrt{\Gamma^* \lambda}$ (eqn 11).

221

222   The CO_2 compensation point, Γ^*, is assumed to be the same for all C_3 species but

223   increases with temperature (Bernacchi *et al*. 2001), suggesting that g_1 should increase

224   with growth temperature. For a given growth temperature, the slope parameter g_1 is

225   determined by the marginal water cost of carbon, λ. To date, it has been unclear how λ

226   varies among species and growth conditions, partly because of the difficulty of

227   quantifying λ using existing methods. The model proposed here offers a new and simple

228   means of quantifying λ, by fitting equation (10) to stomatal conductance measurements

229   and using the fitted parameter g_1 as a proxy for λ.

230   Two key assumptions were needed to derive equation (10). First, the atmospheric CO_2

231   concentration, C_a, is assumed to be much larger than the CO_2 compensation point. The

232   expression breaks down for CO_2 concentrations below approximately 120 µmol mol^{-1}. As

233   the Earth's atmospheric CO_2 concentration has never fallen to this level (Ehleringer *et al*.

234   2007), this assumption does not limit the applicability of the approximation. Second, we

235   assume that stomata behave in such a way that they optimise for RuBP regeneration

236   limited photosynthesis (eqn 8), rather than for Rubisco limited photosynthesis (eqn 9).

237   This assumption is discussed below.

238   **Model testing**

239   We obtained eight datasets of diurnal courses of stomatal conductance measured on field-

240   grown trees from a range of different forest types and climates. We fitted equations (2),

241   (3) and (10) to these datasets using SigmaPlot (v. 11.0, Systat Software Inc.). Table 1

242   gives the details of the datasets used and Table 2 shows the statistics of the model fits.

243   The three models fit the datasets equally well, with no model being consistently better for

244   all datasets (Table 2). R^2 values are similar among models, with the exception of the

245   Duke pine dataset, where the two models based on D gave much better fits than the

246   model based on h_r. Although the models performed similarly across the data sets, the

247   parameter values for the Leuning (1995) model (eqn 3) were not identifiable (i.e. not

248   significantly different from zero) for five of the eight datasets.

249   Fits of the unified model (eqn 10) to the datasets are shown in Figure 2. For this figure,

250   relationships were fitted without the intercept to demonstrate differences in the slope. The

251   key point demonstrated by Figure 2 is that the slope of the relationship clearly differs

252   among species, and varies in a consistent manner. As predicted from equation (11), the

253   slope increases with growth temperature, with slopes highest in tropical savanna species

254   and lowest in Sitka spruce growing in Scotland. Also, although there is some

255   confounding between growth temperature and plant functional type in the datasets

256   presented in Figure 2, we can nevertheless identify clear differences among plant

257   functional types. Slopes were lowest in gymnosperms and highest in angiosperms, and

258   eucalypts have a considerably higher slope than do pines growing at similar latitudes

259   (Tables 1 and 2).

260

261

Discussion

263   We have reconciled two long-standing approaches to modelling stomatal conductance,

264   showing that the theory of optimal stomatal conductance leads to a model with the same

265   form as widely-used empirical models. The unified model has some practical advantages

266   over existing empirical models. It incorporates a dependence on vapour pressure deficit,

267   rather than relative humidity, which agrees better with our mechanistic understanding that

268   stomata respond to transpiration rate (Mott and Parkhurst 1991). Also, the parameters are

269   identifiable from data, so differences in parameters across datasets are meaningful,

270   allowing parameter values to be compared.

271   The most important advantage gained by reconciling the two models, however, is that the

272   slope parameter of the model, which was previously treated as an empirical constant, now

273   has a biological interpretation. This step opens up a way forwards for developing a

274   general theory for variation in stomatal behaviour across species, plant functional types

275   and environments.

276   We show that the slope parameter g_1 is proportional to both the CO_2 compensation point

277   and the marginal water cost of carbon gain (eqn 11; Figure 1b). The CO_2 compensation

278   point depends on temperature according to a well-defined relationship that can be

279   assumed constant for all C_3 species (Bernacchi *et al*. 2001). We predict that, for a given

280   species, the slope parameter g_1 should increase with growth temperature; and that the rate

281   of increase should follow the square root of the temperature-dependence of the CO_2

282   compensation point (eqn 11). The increase in slope with temperature is borne out by the

283   contrast among the sample data sets shown in Figure 2 (see also Table 1). It is also

284   confirmed by a survey of stomatal conductance and stable isotope data across species and

285   environments by Lloyd and Farquhar (1994). These authors derived values analogous to

286   our slope parameter, g_1, and found that values were lower for cold/cool zone vegetation

287   than for warm temperate vegetation, as predicted by our model.

288　The slope parameter g_1 is also related to the marginal water cost of plant carbon gain, λ

289　(mol H_2O mol^{-1} C). The value of λ can be thought of as representing the amount of water

290　that a plant is prepared to spend to gain carbon: a high value of λ indicates "profligate"

291　behaviour while a low value of λ indicates "conservative" behaviour. Cowan and

292　Farquhar (1977) argued that the parameter λ was only likely to remain constant on short

293　time scales, varying from day to day, and this perception has limited the use of the model

294　in the past. However, the fact that functionally equivalent empirical models have been

295　successfully applied using constant parameter values strongly suggests that the value of λ

296　is stable on longer time scales, making it an informative parameter. Theoretical studies of

297　λ, and experimental studies using the empirical models, indicate two major sources of

298　variation in λ: differences among species, related to whole-plant water-use strategy, and

299　effects of low soil moisture availability.

300　Theoretical work suggests that λ is likely to be related to whole-plant carbon-water

301　economy (Givnish 1986). Our comparison among ecosystems (Figure 2) provides clear

302　evidence for differences in stomatal behaviour among plant functional types, indicating a

303　link with whole-plant traits. The contrast that we found between angiosperms and

304　gymnosperms, with angiosperms having higher values of λ, is strongly supported by the

305　cross-species survey by Lloyd and Farquhar (1994). The slope parameter of the empirical

306　stomatal models also varies among species in a way that appears linked to plant water use

307　strategy (e.g. Medlyn *et al*. 2001). Furthermore, evidence is accumulating that

308　photosynthetic capacity and maximal stomatal conductance are related to plant hydraulic

309　architecture (e.g. Nardini and Salleo 2000, Clearwater and Meinzer 2001, Hubbard *et al.*

310　2001, Katul *et al*. 2003, Mencuccini 2003, Bucci *et al*. 2005, Taylor and Eamus 2008).

311　Thus, values of λ obtained under well-watered conditions are likely to be a useful

312　quantitative way of characterizing whole-plant-level water-use strategies.

313　Under drought conditions, theoretical analysis of the optimal stomatal conductance

314　indicates that the expected value of carbon assimilation is maximised if the value of λ

315　declines as drought progresses, at a rate determined by the probability of rain on any

316　given day (Mäkelä *et al.* 1996). Some models that use the empirical approach incorporate

317   an equivalent assumption, reducing the slope parameter g_1 as a function of soil moisture

318   content (e.g. Sala and Tenhunen 1996, Kirschbaum 1999). Some recent implementations

319   decrease the slope parameter as a function of leaf water potential rather than soil moisture

320   content (e.g. Tuzet *et al*. 2003). Such assumptions have been found to improve

321   simulations of forest water use during drought (e.g. Sala and Tenhunen 1996), and of

322   leaf-level photosynthesis and transpiration over a growing season (Berninger *et al*. 1996,

323   Op de Beeck *et al*. 2010). However, very few studies have directly examined how the

324   relationship between photosynthesis and stomatal conductance is affected by drought.

325   One study on *Pinus ponderosa* that directly examined this question found that the model

326   intercept, rather than the slope, was related to soil moisture potential (Misson *et al.* 2004).

327   It can be questioned whether the optimization criterion assumed here (eqn 4) can still be

328   said to be optimal if drought stress starts to threaten plant survival. It may be that the

329   relationship given by eqn (10) will break down as soil moisture potential is reduced.

330   Nonetheless, eqn (10) offers a quantitative framework within which it would be possible

331   to critically examine how soil moisture stress affects stomatal behaviour.

332   By linking the optimal and empirical stomatal models, we have identified a new and

333   simple way of estimating λ from measurements of stomatal conductance. We suggest that

334   comparative studies of such values λ across species and soil moisture conditions are

335   likely to bring new insights into adaptation of stomatal behaviour and plant water-use

336   strategies.

337   *Response to atmospheric CO$_2$ concentration*

338   One of the major assumptions required by our derivation was that stomatal conductance

339   acts as if it is optimizing for RuBP-regeneration-limited photosynthesis (eqn 8), rather

340   than Rubisco-limited photosynthesis (eqn 9).Importantly, this is not the same as assuming

341   that photosynthesis is always limited by RuBP-regeneration; we only assume that stomata

342   behave as if it were. We justify this assumption as follows. Firstly, stomatal responses to

343   CO$_2$ can be observed in epidermal peels, indicating that the CO$_2$ sensing mechanism

344   resides in the guard cells per se, not in the mesophyll (Travis and Mansfield 1979,

345   Assmann 1999). However, although guard cells have a significant capacity for electron

346 transport, they have a relatively low capacity for Rubisco C fixation (e.g. Outlaw et al.

347 1979, Shimazaki 1989, Outlaw and DeVlieghere-He 2001). Thus, while it is plausible

348 that stomatal behaviour could be regulated by rates of electron transport, it seems

349 implausible that stomatal behaviour would be regulated by rates of C fixation, or the

350 balance between the two processes. Secondly, RuBP regeneration plays a role in limiting

351 photosynthesis under most environmental conditions: it is the major limitation in leaves

352 below light saturation and it tends to co-limit photosynthesis in light-saturated leaves

353 (Farquhar *et al*. 1980, Woodrow 1994). In exploratory simulations using the full

354 numerical solution of Arneth et al. (2002), we calculated annual water use efficiency

355 (WUE) for needles growing in a pine canopy under the assumptions that stomatal

356 behaviour was optimized for (i) RuBP regeneration limited photosynthesis, (ii) Rubisco

357 limited photosynthesis, or (iii) whichever of the two processes was most limiting to

358 photosynthesis at any one time point. The difference in WUE between simulations (i) and

359 (iii) was of the order of 1% for a range of parameter values, whereas WUE under

360 simulation (ii) was 50-75% lower than that of simulation (iii). These results suggest that

361 stomatal behaviour which optimizes as if RuBP regeneration were limiting to

362 photosynthesis is very close to the theoretical optimal behaviour, resulting in little

363 evolutionary pressure to achieve the theoretical optimum. Finally, these conclusions are

364 further supported by the observation that the correlation between photosynthesis and

365 stomatal conductance breaks down in transgenic plants with impaired Rubisco activity

366 (von Caemmerer et al. 2004). Reduced Rubisco activity reduces photosynthetic capacity

367 in such plants, but does not appear to impact on stomatal conductance or its

368 responsiveness to C_a.

369 Importantly, when we make this assumption, the resulting model correctly captures the

370 observed response to atmospheric CO_2 concentration (C_a). The response to C_a predicted

371 by the optimal stomatal model differs considerably according to which limitation is

372 considered, as shown in Figure 3. If Rubisco-limited photosynthesis is considered,

373 stomatal conductance is predicted to increase with increased C_a, contrary to extensive

374 experimental observations (see Morison 1987 for review). In contrast, if RuBP-

375 regeneration-limited photosynthesis is considered, stomatal conductance is predicted to

15

376   decline non-linearly with C_a, which agrees closely with observations (Morison 1987).

377   This assumption thus allows the model to be used to investigate responses to rising C_a.

378   For example, Katul *et al.* (2009b) recently applied the optimal stomatal conductance

379   model to datasets from a large-scale CO_2 enrichment study, the Duke FACE experiment.

380   They estimated λ from ambient and enriched CO_2 treatments, and concluded that this

381   parameter differs between treatments. However, they assumed Rubisco-limited

382   photosynthesis throughout their study, and their conclusion is thus driven by the use of a

383   model with an incorrect short-term C_a response. In contrast, we fitted our eqn (10) to an

384   expanded dataset with ten years of data from the same FACE experiment, and found that

385   there was no effect of CO_2 treatment on the value of λ (Figure 4). Therefore, because

386   there is no change in the parameter value between treatments, we can conclude that there

387   was no acclimation of stomatal conductance to CO_2 enrichment in this FACE experiment

388   (cf. Medlyn *et al.* 2001)

389   *Response to D*

390   The response to vapour pressure deficit (*D*) predicted by the optimal stomatal model was

391   investigated by Katul *et al.* (2009a). They showed that the predicted *D* response is

392   consistent with observations and also quite consistent with the *D* response of the Leuning

393   (1995) empirical model (eqn 3) over the normal operating range of *D*. We note that

394   Leuning (1995) considered alternative forms for the *D* response, but specifically did not

395   consider the form $D^{-1/2}$, despite observing that Lloyd (1991) had found this function to

396   give the best fit to data from *Macadamia integrifolia*. The major difference between the

397   hyperbolic *D* response used in the Leuning model (eqn 3) and the square root

398   dependence given by the optimal stomatal model (e.g. eqn 10) lies in the behaviour of g_s

399   as *D* approaches zero. Stomatal conductance at low *D* is bounded in equation (3) but

400   unbounded in equation (10). However, an unbounded g_s at low *D* should not be seen as a

401   problem. There is evidence from eddy covariance studies to suggest that stomatal

402   conductance is in fact unbounded as VPD approaches zero (Wang *et al.* 2009), supporting

403   the *D* response emerging from the unified model. Also, although g_s may be unbounded,

404   transpiration (E) is not; $E \approx g_s * D$, so that $E \propto \sqrt{D}$ using the unified stomatal model.

405　Thus, an unbounded value of g_s is acceptable, from viewpoints of both model correctness

406　and model stability.

407

Conclusion

408

409　We have reconciled two long-standing theories for stomatal conductance. We combined

410　Cowan & Farquhar (1977)'s theory of optimal stomatal behaviour with the Farquhar *et*

411　*al*. (1980) model of photosynthesis, and derived a new model expression for stomatal

412　conductance that has the same form as current empirical models. The unified model thus

413　combines existing experimental evidence with an accepted theory for stomatal behaviour.

414　The model has significant potential to act as a framework for interpreting stomatal

415　behaviour among species across a range of environmental conditions, including rising

416　atmospheric [CO_2], and to improve simulations of vegetation water use at large scales.

417

423

References

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425　**Ainsworth EA, Rogers A. 2007.** The response of photosynthesis and stomatal

426　conductance to rising [CO_2]: mechanisms and environmental interactions. *Plant*

427　*Cell and Environment* **30**(3): 258-270.

428　**Aphalo PJ, Jarvis PG. 1991.** Do stomata respond to relative humidity? *Plant Cell and*

429　*Environment* **14**(1): 127-132.

430   **Arneth A, Lloyd J, Santruckova H, Bird M, Grigoryev S, Kalaschnikov YN,**
431   **Gleixner G, Schulze ED. 2002.** Response of central Siberian Scots pine to soil
432   water deficit and long-term trends in atmospheric CO_2 concentration. *Global*
433   *Biogeochemical Cycles* **16**(1).

434   **Assmann SM. 1999.** The cellular basis of guard cell sensing of rising CO_2. *Plant Cell*
435   *and Environment* **22**(6): 629-637.

436   **Ball JT, Woodrow IE, Berry JA 1987.** A model predicting stomatal conductance and
437   its contribution to the control of photosynthesis under different environmental
438   conditions. In: Biggins J ed. *Progress in Photosynthesis Research*. Dordrecht, The
439   Netherlands: Martinus-Nijhoff Publishers, 221-224.

440   **Barton CVM, Jarvis PG. 1999.** Growth response of branches of *Picea sitchensis* to four
441   years exposure to elevated atmospheric carbon dioxide concentration. *New*
442   *Phytologist* **144**(2): 233-243.

443   **Bernacchi CJ, Singsaas EL, Pimentel C, Portis AR, Long SP. 2001.** Improved
444   temperature response functions for models of Rubisco-limited photosynthesis.
445   *Plant Cell and Environment* **24**(2): 253-259.

446   **Berninger F, Makela A, Hari P. 1996.** Optimal control of gas exchange during drought:
447   Empirical evidence. *Annals of Botany* **77**(5): 469-476.

448   **Bonan G. 2008.** *Ecological Climatology.* 2nd ed. Cambridge University Press,
449   Cambridge.

450   **Bucci SJ, Goldstein G, Meinzer FC, Franco AC, Campanello P, Scholz FG. 2005.**
451   Mechanisms contributing to seasonal homeostasis of minimum leaf water
452   potential and predawn disequilibrium between soil and plant water potential in
453   neotropical savanna trees. *Trees-Structure and Function* **19**(3): 296-304.

454   **Clearwater MJ, Meinzer FC. 2001.** Relationships between hydraulic architecture and
455   leaf photosynthetic capacity in nitrogen-fertilized Eucalyptus grandis trees. *Tree*
456   *Physiology* **21**(10): 683-690.

457   **Cowan IR, Farquhar GD 1977.** Stomatal function in relation to leaf metabolism and
458   environment. In: Jennings DH ed. *Integration of activity in the higher plant*.
459   Cambridge: Cambridge University Press, 471-505.

460 **Cox PM 2001**. Description of the "TRIFFID" Dynamic Global Vegetation Model.

461   Hadley Centre Technical Note 24. URL :

462   http://climate.uvic.ca/common/HCTN_24.pdf.

463 **Crous KY, Ellsworth DS 2004.** Canopy position affects photosynthetic adjustments to

464   long-term elevated CO_2 concentration (FACE) in aging needles in a mature Pinus

465   taeda forest. *Tree Physiology* **24**(9): 961-970.

466 **Crous KY, Walters MB, Ellsworth DS. 2008.** Elevated CO_2 concentration affects leaf

467   photosynthesis-nitrogen relationships in *Pinus taeda* over nine years in FACE.

468   *Tree Physiology* **28**(4): 607-614.

469 **Eamus D, Taylor DT, Macinnis-Ng CMO, Shanahan, S and de Silva, L 2008**.

470   Comparing model predictions and experimental data for the response of stomatal

471   conductance and guard cell turgor to manipulations of cuticular conductance, leaf-

472   to-air vapour pressure difference and temperature: feedback mechanisms are able

473   to account for all observations. *Plant Cell and Environment* **31**, 269 – 277.

474 **Ehleringer JR, Cerling T, Dearing MD 2007.** Preface. In: Ehleringer JR, Cerling T,

475   Dearing MD eds. *A History of Atmospheric CO_2 and Its Effects on Plants,*

476   *Animals, and Ecosystems*. New York, NY, USA: Springer, v-vii.

477 **Ellsworth D. 2000.** Seasonal CO_2 assimilation and stomatal limitations in a *Pinus taeda*

478   canopy. *Tree Physiology* **20**: 435-445.

479 **Ellsworth DS, Reich PB, Naumburg ES, Koch GW, Kubiske ME, Smith SD. 2004.**

480   Photosynthesis, carboxylation and leaf nitrogen responses of 16 species to

481   elevated pCO(2) across four free-air CO_2 enrichment experiments in forest,

482   grassland and desert. *Global Change Biology* **10**(12): 2121-2138.

483 **Ellsworth DS. 1999.** CO_2 enrichment in a maturing pine forest: are CO_2 exchange and

484   water status in the canopy affected? *Plant Cell and Environment* **22**(5): 461-472.

485 **Farquhar GD, Von Caemmerer S, Berry JA. 1980.** A biochemical model of

486   photosynthetic carbon dioxide assimilation in leaves of 3-carbon pathway species.

487   *Planta* **149**(1): 78-90.

488 **Freeman M. 1998.** *Leaf gas exchange in mature beech (Fagus sylvatica L.) exposed to*

489   *long-term elevated CO_2 in branch bags.* PhD thesis, Royal Veterinary and

490   Agricultural University, Denmark.

491 **Givnish TJ 1986.** Optimal stomatal conductance, alocation of energy between leaves and
492   roots, and the marginal cost of transpiration. In: Givnish TJ ed. *On the Economy*
493   *of Plant Form and Function*: Cambridge University Press, 171 - 213.
494 **Hari P, Mäkelä A, Berninger F, Pohja T. 1999.** Field evidence for the optimality
495   hypothesis of gas exchange in plants. *Australian Journal of Plant Physiology*
496   **26**(3): 239-244.
497 **Hari P, Mäkelä A, Korpilahti E, Holmberg M. 1986.** Optimal control of gas exchange.
498   *Tree Physiology* **2**(1-3): 169-175.
499 **Hubbard RM, Ryan MG, Stiller V, Sperry JS. 2001.** Stomatal conductance and
500   photosynthesis vary linearly with plant hydraulic conductance in ponderosa pine.
501   *Plant Cell and Environment* **24**(1): 113-121.
502 **Kattge J, Knorr W, Raddatz T, Wirth C. 2009.** Quantifying photosynthetic capacity
503   and its relationship to leaf nitrogen content for global-scale terrestrial biosphere
504   models. *Global Change Biology* **15**(4): 976-991.
505 **Kattge J, Knorr W. 2007.** Temperature acclimation in a biochemical model of
506   photosynthesis: a reanalysis of data from 36 species. *Plant Cell and Environment*
507   **30**(9): 1176-1190.
508 **Katul G, Ellsworth D, Lai CT. 2000.** Modelling assimilation and intercellular CO_2 from
509   measured conductance: a synthesis of approaches. *Plant, Cell & Environment* **23**:
510   1313-1328.
511 **Katul G, Leuning R, Oren R. 2003.** Relationship between plant hydraulic and
512   biochemical properties derived from a steady-state coupled water and carbon
513   transport model. *Plant Cell and Environment* **26**(3): 339-350.
514 **Katul GG, Palmroth S, Oren R. 2009a.** Leaf stomatal responses to vapour pressure
515   deficit under current and CO_2-enriched atmosphere explained by the economics of
516   gas exchange. *Plant Cell and Environment* **32**(8): 968-979.
517 **Katul G, Manzoni S, Palmroth S, Oren R 2009b.** A stomatal optimization theory to
518   describe the effects of atmospheric CO_2 on leaf photosynthesis and transpiration.
519   *Annals of Botany* doi:10.1093/aob/mcp292.
520 **Kirschbaum MUF. 1999.** CenW, a forest growth model with linked carbon, energy,
521   nutrient and water cycles. *Ecological Modelling* **118**(1): 17-59.

522   **Kolari P, Lappalainen HK, Hanninen H, Hari P. 2007.** Relationship between
523   temperature and the seasonal course of photosynthesis in Scots pine at northern
524   timberline and in southern boreal zone. *Tellus Series B-Chemical and Physical*
525   *Meteorology* **59**(3): 542-552.
526   **Krinner G, Viovy N, de Noblet-Ducoudre N, Ogee J, Polcher J, Friedlingstein P,**
527   **Ciais P, Sitch S, Prentice IC. 2005.** A dynamic global vegetation model for
528   studies of the coupled atmosphere-biosphere system. *Global Biogeochemical*
529   *Cycles* **19**(1).
530   **Law RM, Kowalczyk EA, Wang YP. 2006.** Using atmospheric CO_2 data to assess a
531   simplified carbon-climate simulation for the 20th century. *Tellus Series B-*
532   *Chemical and Physical Meteorology* **58**(5): 427-437.
533   **Leuning R. 1995.** A critical appraisal of a coupled stomatal-photosynthesis model for C_3
534   plants. *Plant Cell and Environment* **18**: 339-357.
535   **Lloyd J. 1991.** Modeling stomatal responses to environment in *Macadamia integrifolia*.
536   *Australian Journal of Plant Physiology* **18**(6): 649-660.
537   **Lloyd J, Farquhar GD. 1994.** C-13 Discrimination during CO_2 assimilation by the
538   terrestrial biosphere. *Oecologia* **99**(3-4): 201-215.
539   **Mäkelä A, Berninger F, Hari P. 1996.** Optimal control of gas exchange during drought:
540   Theoretical analysis. *Annals of Botany* **77**(5): 461-467.
541   **Mäkelä A, Hari P, Berninger F, Hanninen H, Nikinmaa E. 2004.** Acclimation of
542   photosynthetic capacity in Scots pine to the annual cycle of temperature. *Tree*
543   *Physiology* **24**(4): 369-376.
544   **Mäkelä A, Kolari P, Karimaki J, Nikinmaa E, Peramaki M, Hari P. 2006.** Modelling
545   five years of weather-driven variation of GPP in a boreal forest. *Agricultural and*
546   *Forest Meteorology* **139**(3-4): 382-398.
547   **Medlyn BE, Badeck FW, De Pury DGG, Barton CVM, Broadmeadow M,**
548   **Ceulemans R, De Angelis P, Forstreuter M, Jach ME, Kellomaki S, Laitat E,**
549   **Marek M, Philippot S, Rey A, Strassemeyer J, Laitinen K, Liozon R, Portier**
550   **B, Roberntz P, Wang K, Jarvis PG. 1999.** Effects of elevated [CO_2] on
551   photosynthesis in European forest species: a meta-analysis of model parameters.
552   *Plant Cell and Environment* **22**(12): 1475-1495.

553 **Medlyn BE, Barton CVM, Broadmeadow MSJ, Ceulemans R, De Angelis P,**
554   **Forstreuter M, Freeman M, Jackson SB, Kellomäki S, Laitat E, Rey A,**
555   **Roberntz P, Sigurdsson BD, Strassemeyer J, Wang K, Curtis PS, Jarvis PG.**
556   **2001.** Stomatal conductance of forest species after long-term exposure to elevated
557   CO_2 concentration: a synthesis. *New Phytologist* **149**(2): 247-264.

558 **Medlyn BE, Berbigier P, Clement R, Grelle A, Loustau D, Linder S, Wingate L,**
559   **Jarvis PG, Sigurdsson BD, McMurtrie RE. 2005.** Carbon balance of coniferous
560   forests growing in contrasting climates: Model-based analysis. *Agricultural and*
561   *Forest Meteorology* **131**(1-2): 97-124.

562 **Medlyn BE, Dreyer E, Ellsworth D, Forstreuter M, Harley PC, Kirschbaum MUF,**
563   **LeRoux X, Loustau D, Montpied P, Strassemeyer J, Walcroft A, Wang K.**
564   **2002.** Temperature response of parameters of a biochemically-based model of
565   photosynthesis. II. A review of experimental data. *Plant Cell and Environment*
566   **25**: 1167-1179.

567 **Medlyn BE, Pepper DA, O'Grady AP, Keith H. 2007.** Linking leaf and tree water use
568   with an individual-tree model. *Tree Physiology* **27**(12): 1687-1699.

569 **Mencuccini M. 2003.** The ecological significance of long-distance water transport: short-
570   term regulation, long-term acclimation and the hydraulic costs of stature across
571   plant life forms. *Plant Cell and Environment* **26**(1): 163-182.

572 **Misson L, Panek JA, Goldstein AH. 2004.** A comparison of three approaches to
573   modeling leaf gas exchange in annually drought-stressed ponderosa pine forests.
574   *Tree Physiology* **24**(5): 529-541.

575 **Morison JIL 1987.** Intercellular CO_2 concentration and stomatal response to CO_2. In:
576   Zeiger E, Cowan IR, Farquhar GD, eds. *Stomatal function*. Stanford University
577   Press, pp229-251.

578 **Mott KA, Parkhurst DF. 1991.** Stomatal responses to humidity in air and helox. *Plant*
579   *Cell and Environment* **14**(5): 509-515.

580 **Nardini A, Salleo S. 2000.** Limitation of stomatal conductance by hydraulic traits:
581   sensing or preventing xylem cavitation? *Trees-Structure and Function* **15**(1): 14-
582   24.

583   **Niinemets U, Diaz-Espejo A, Flexas J, Galmes J, Warren CR. 2009.** Importance of
584   mesophyll diffusion conductance in estimation of plant photosynthesis in the
585   field. *Journal of Experimental Botany* **60**(8): 2271-2282.

586   **Op de Beeck M, Low M, Deckmyn G, Ceulemans R.** A comparison of photosynthesis-
587   dependent stomatal models using twig cuvette field data for adult beech (Fagus
588   sylvatica L.). *Agricultural and Forest Meteorology* **150**(4): 531-540.

589   **Oleson K, Dai Y, Bonan G, Bosilovoch M, Dickinson R, Dirmeyer P, Hoffman F,**
590   **Houser P, Levis S, Niu G 2004.** Technical description of the community land
591   model (CLM). Tech. Note NCAR/TN-461+ STR.

592   **Ostle NJ, Smith P, Fisher R, Woodward FI, Fisher JB, Smith JU, Galbraith D, Levy**
593   **P, Meir P, McNamara NP, Bardgett RD. 2009.** Integrating plant-soil
594   interactions into global carbon cycle models. *Journal of Ecology* **97**(5): 851-863.

595   **Outlaw WH, Manchester J, DiCamelli CA, Randall DD, Rapp B, Veith GM. 1979.**
596   Photosynthetic carbon reduction pathway is absent in chloroplasts of *Vicia faba*
597   guard cells. *Proceedings of the National Academy of Sciences of the United States*
598   *of America* **76**(12): 6371-6375.

599   **Outlaw WH, De Vlieghere-He X. 2001.** Transpiration rate. An important factor
600   controlling the sucrose content of the guard cell apoplast of broad bean. *Plant*
601   *Physiology* **126**(4): 1716-1724.

602   **Piao SL, Friedlingstein P, Ciais P, de Noblet-Ducoudre N, Labat D, Zaehle S. 2007.**
603   Changes in climate and land use have a larger direct impact than rising CO_2 on
604   global river runoff trends. *Proceedings of the National Academy of Sciences of the*
605   *United States of America* **104**(39): 15242-15247.

606   **Pitman AJ. 2003.** The evolution of, and revolution in, land surface schemes designed for
607   climate models. *International Journal of Climatology* **23**(5): 479-510.

608   **Sala A, Tenhunen JD. 1996.** Simulations of canopy net photosynthesis and transpiration
609   in *Quercus ilex* L. under the influence of seasonal drought. *Agricultural and*
610   *Forest Meteorology* **78**: 203-222.

611   **Scarascia-Mugnozza GE, De Angelis P, Matteuci G, Kuzminsky E 1996.** Carbon
612   metabolism and plant growth under elevated CO_2 in a natural *Quercus ilex*

613   macchia stand. In: Koch GW, Mooney HA eds. *Carbon Dioxide and Terrestrial Ecosystems*. San Diego: Academic Press, 209-230.

Scholze M, Knorr W, Arnell NW, Prentice IC. 2006. A climate-change risk analysis for world ecosystems. *Proceedings of the National Academy of Sciences of the United States of America* **103**(35): 13116-13120.

Sellers PJ, Dickinson RE, Randall DA, Betts AK, Hall FG, Berry JA, Collatz GJ, Denning AS, Mooney HA, Nobre CA, Sato N, Field CB, HendersonSellers A. 1997. Modeling the exchanges of energy, water, and carbon between continents and the atmosphere. *Science* **275**(5299): 502-509.

Shimazaki K. 1989. Ribulose bisphosphate carboxylase activity and photosynthetic O_2 evolution rate in *Vicia* guard-cell protoplasts. *Plant Physiology* **91**(2): 459-463.

Sitch S, Huntingford C, Gedney N, Levy PE, Lomas M, Piao SL, Betts R, Ciais P, Cox P, Friedlingstein P, Jones CD, Prentice IC, Woodward FI. 2008. Evaluation of the terrestrial carbon cycle, future plant geography and climate-carbon cycle feedbacks using five Dynamic Global Vegetation Models (DGVMs). *Global Change Biology* **14**(9): 2015-2039.

Sitch S, Smith B, Prentice IC, Arneth A, Bondeau A, Cramer W, Kaplan JO, Levis S, Lucht W, Sykes MT, Thonicke K, Venevsky S. 2003. Evaluation of ecosystem dynamics, plant geography and terrestrial carbon cycling in the LPJ dynamic global vegetation model. *Global Change Biology* **9**(2): 161-185.

Taylor D, Eamus D. 2008. Coordinating leaf functional traits with branch hydraulic conductivity: resource substitution and implications for carbon gain. *Tree Physiology* **28**(8): 1169-1177.

Thomas DS, Eamus D, Bell D. 1999. Optimization theory of stomatal behaviour - I. A critical evaluation of five methods of calculation. *Journal of Experimental Botany* **50**(332): 385-392.

Thomas DS, Eamus D. 2002. Seasonal patterns of xylem sap pH, xylem abscisic acid concentration, leaf water potential and stomatal conductance of six evergreen and deciduous Australian savanna tree species. *Australian Journal of Botany* **50**(2): 229-236.

643   **Travis AJ, Mansfield TA. 1979.** Stomatal responses to light and CO_2 are dependent on

644   KCl concentration. *Plant Cell and Environment* **2**: 319-323.

645   **Tuzet A, Perrier A, Leuning R. 2003.** A coupled model of stomatal conductance,

646   photosynthesis and transpiration. *Plant Cell and Environment* **26**(7): 1097-1116.

647   **von Caemmerer S, Lawson T, Oxborough K, Baker NR, Andrews TJ, Raines CA.**

648   **2004.** Stomatal conductance does not correlate with photosynthetic capacity in

649   transgenic tobacco with reduced amounts of Rubisco. *Journal of Experimental*

650   *Botany* **55**(400): 1157-1166.

651   **Wang SS, Yang Y, Trishchenko AP. 2009.** Assessment of canopy stomatal conductance

652   models using flux measurements. *Ecological Modelling* **220**(17): 2115-2118.

653   **Wingate L, Seibt U, Moncrieff JB, Jarvis PG, Lloyd J. 2007.** Variations in C-13

654   discrimination during CO2 exchange by Picea sitchensis branches in the field.

655   *Plant Cell and Environment* **30**(5): 600-616.

656   **Wong SC, Cowan IR, Farquhar GD. 1979.** Stomatal conductance correlates with

657   photosynthetic capacity. *Nature* **282**(22 November): 424-426.

658   **Woodrow IE. 1994.** Optimal acclimation of the C_3 photosynthetic system under

659   enhanced CO_2. *Photosynthesis Research* **39**(3): 401-412.

660   **Wullschleger SD. 1993.** Biochemical limitations to carbon assimilation in C_3 plants - a

661   retrospective analysis of the *A/C_i* curves from 109 species. *Journal of*

662   *Experimental Botany* **44**: 907-920.

663   **Zeppel M, Macinnis-Ng C, Palmer A, Taylor D, Whitley R, Fuentes S, Yunusa I,**

664   **Williams M, Eamus D. 2008.** An analysis of the sensitivity of sap flux to soil and

665   plant variables assessed for an Australian woodland using a soil-plant-atmosphere

666   model. *Functional Plant Biology* **35**(6): 509-520.

667

668

669

670 **Appendix: Derivation of analytical approximation to the optimal stomatal control**

671 **model**

672

673 In their Appendix, Arneth *et al.* (2002) describe how the optimal stomatal control model

674 can be combined with the Farquhar – von Caemmerer model of leaf photosynthesis to

675 obtain two quadratic expressions for the optimal C_i. Different expressions are obtained

676 according to whether Rubisco activity, or RuBP regeneration, is limiting photosynthesis.

677 In what follows, we focus on the case where RuBP regeneration is the limiting factor. We

678 make this assumption because RuBP regeneration is limiting at low light levels, and at

679 high light levels the two factors tend to co-limit photosynthesis. We consider this

680 assumption further in the discussion.

681

682 Arneth *et al.* (2002) assumed leaf dark respiration $R_d = 0$. We also make this assumption

683 to make the derivation below clearer. Equivalent expressions can be derived for the case

684 where $R_d > 0$, but the resulting simple model is identical to equation (10). Under this

685 assumption, for RuBP-regeneration limited photosynthesis, the optimal C_i is given by a

686 root of the quadratic $a C_i^2 + b C_i + c$, where:

687

688 $\qquad a = 3\,\Gamma^* - L$

689

690 $\qquad b = 2\,\Gamma^*\,(L - 3\,C_a)$ $\qquad\qquad\qquad\qquad\qquad$ (A1)

691

692 $\qquad c = L\,(2\,\Gamma^{*2}\,(1 - 3\,C_a\,\Gamma^*) + 3\,C_a^2\,\Gamma^*$

693

694 where *L* represents the combination of terms (= $1.6\,D\,/\,\lambda$). The discriminant, $\Delta = b^2 - 4\,a$

695 *c*, can be calculated to be:

696

697 $\qquad \Delta = 12\,\Gamma^*\,L\,(C_a^2 + C_a\,\Gamma^* - C_a\,L + \Gamma^*\,L - 2\,\Gamma^{*2})$ $\qquad\qquad$ (A2)

698

699 If we assume that $C_a \gg \Gamma^*$, this expression simplifies to

700

26

701 $$\Delta \approx 12\,\Gamma^* L\,C_a^2 \tag{A3}$$

702

703 The solution to the quadratic is then

704

705 $$C_i = \frac{3C_a\Gamma^* - \Gamma^* L - C_a\sqrt{3\Gamma^* L}}{3\Gamma^* - L} \tag{A4}$$

706

707 We are seeking an expression of the form

708

709 $$g_s = f\,A/\,C_a \tag{A5}$$

710

711 Rearranging (A5), we obtain

712

713 $$f\ = g_s\,C_a\,/\,A = C_a\,/\,(C_a - C_i) \tag{A6}$$

714

715 Substituting in optimal C_i from equation (A4), we obtain

716

717 $$f \approx \frac{3\Gamma^* - L}{\sqrt{3\Gamma^* L} - L + \Gamma^* L/C_a} \tag{A7}$$

718

719 Assuming that $\Gamma^* L \ll C_a$, this simplifies to

720

721 $$f \approx \frac{3\Gamma^* - L}{\sqrt{3\Gamma^* L} - L} \tag{A8}$$

722

723 And, by completing the square in the numerator, thence to

724

725 $$f \approx 1 + \sqrt{\frac{3\Gamma^*}{L}} \tag{A9}$$

726

727 Combining expressions (A5) and (A9), we obtain the following expression for optimal

728 stomatal conductance:

729

730 $$g_s^* \ = \ (1+\sqrt{\frac{3\Gamma^*\lambda}{1.6D}}) \ \frac{A}{C_a}$$ (A10)

731

732 Inspection of this equation shows that the optimal stomatal conductance is proportional to

733 assimilation rate, inversely proportional to C_a, and approximately inversely proportional

734 to the square root of D. Thus, equation (A10) simplifies to:

735 $$g_s^* = g_0 + g_1 \frac{A}{C_a\sqrt{D}}$$ (A11)

736

737 where the slope parameter, g_1, is linearly related to the parameter combination $\sqrt{\Gamma^*\lambda}$.

738

739

Figure Captions

741   Figure 1. Test of the accuracy of the approximate model. (a) A comparison of the

742   approximate solution (Eq 10) and the exact numerical solution to the optimal stomatal

743   model coupled with the Farquhar *et al*. (1980) model of photosynthesis. To generate this

744   figure, the following environmental drivers were varied factorially: PAR (50 – 1550 μmol

745   $m^{-2} s^{-1}$), relative humidity (30 – 80%), C_a (320 – 700 ppm). For several different values of

746   λ, equation (10) was fitted to output from the numerical model and the corresponding

747   slope g_1 obtained. This slope was then used to estimate the optimal g_s from the

748   environmental drivers, using equation (10). (b) The relationship between the slope

749   parameter g_1 and the parameter combination $\sqrt{\Gamma^* \lambda}$. To generate this figure, optimal

750   stomatal conductance was solved numerically for a range of λ values, each time varying

751   the same environmental drivers as in Figure 1(a). For each value of λ, the slope parameter

752   g_1 was found by fitting Eq 11 to the simulation results.

753   Figure 2. The unified stomatal model (eqn 10) fitted to eight datasets from contrasting

754   forest ecosystems. Details of the ecosystems are given in Table 1. Blue shades show data

755   from conifers, green shows data from deciduous angiosperms, and red/purple shades

756   show data from broadleaf evergreen forests. For this figure, the model was fitted fixing

757   the intercept to zero. Fitted slopes were as follows: Sitka A, 4.2; Sitka B, 4.7; Duke Pine,

758   6.1; Fagus, 6.8; Alpine Ash, 7.1; Macchia, 9.8; Savanna, 12.5; Red Gum, 15.1.

759   Figure 3. The response of stomatal conductance (g_s) to atmospheric CO_2 concentration

760   (C_a) predicted by the full numerical solution to the optimal stomatal model. Solid line:

761   coupled with RuBP-regeneration limited photosynthesis (eqn 8); Dashed line: coupled

762   with Rubisco-limited photosynthesis (eqn 9).

763   Figure 4. The unified stomatal model (eqn 10) fitted to data from the Duke FACE

764   experiment. Solid symbols and solid line: data from ambient CO_2 treatment; open

765   symbols and dashed line: data from elevated CO_2 treatment. Regression lines are not

766   significantly different ($p > 0.05$). Data are from spot measurements of pine needle gas

767   exchange at ambient and elevated CO_2 as described in detail in Ellsworth (2000) and

768   Katul *et al*. (2000) for the first 3 years of the Duke FACE experiment, and from spot

769   measurements extracted from complete photosynthetic CO_2 response curves for

770   unfertilised trees from Crous *et al*. (2004) and Crous *et al*. (2008) from the 3rd though 9th

771   years of CO_2 exposure in FACE.

772

773

Tables

Table 1. Details of example data sets used for model testing. Temperature refers to the average leaf temperature at which measurements were taken, and does not necessarily reflect growth temperature. At each site, data from different species were pooled where stomatal behaviour was not distinguishable between species.

Dataset	Species	Location	Lat / Long	Temperature (°C)	Reference
Sitka A	*Picea sitchensis*	Aberfeldy, Scotland	56° 37' N 3° 48' W	13.5	Wingate *et al*. (2007), Medlyn *et al*. (2005)
Sitka B	*Picea sitchensis*	Glencorse, Scotland	55° 31' N 3° 12' W	24.8	Barton & Jarvis (1999), Medlyn *et al*. (2001)
Fagus	*Fagus sylvatica*	Grib Skov, Denmark	55° 59'N 12° 16'E	27.2	Freeman (1998), Medlyn *et al*. (2001)
Duke Pine	*Pinus taeda*	North Carolina, USA	35° 59'N 79° 06'W	28.1	Ellsworth (1999), Ellsworth *et al*. (2004), Crous *et al*. (2008)
Macchia	*Phillyrea angustifolia; Pistacia lentiscus*	Montalto di Castro, Italy	42° 22' N 11° 32' E	32.7	Scarascia Mugnozza *et al*. (1996) Medlyn *et al*. (2001)
Alpine Ash	*Eucalyptus delegatensis*	Snowy Mts, NSW, Australia	35° 39' S 148° 56' E	20.6	Medlyn *et al*. (2007)

Savanna	*6 tropical savanna species*	Darwin, NT, Australia	12° 29' S 130° 59' E	33.0	Thomas & Eamus (2002)
Parramatta Red Gum	*Eucalyptus parramattensis*	Western Sydney, NSW, Australia	33° 39'S 150° 46'E	32.1	Zeppel *et al.* (2008)

Table 2. Statistics of fits of the three alternative models to example data sets. Parameter standard errors are shown in brackets. Units are: g_0, mol m^{-2} s^{-1}; g_1, dimensionless; D_0, kPa.

* indicates parameters that are not significantly different from zero.

Dataset	Eq. 2 (Ball *et al*. 1987)			Eq. 3 (Leuning 1995)				Eq. 11 (this paper)		
	g_0	g_1	R^2	g_0	g_1	D_0	R^2	g_0	g_1	R^2
Sitka A	0.039 (0.004)	4.55 (0.38)	0.651	0.038 (0.003)	7.35 (0.92)	0.35 (0.08)	0.724	0.037 (0.003)	2.10 (0.14)	0.754
Sitka B	0.027 (0.008)	5.17 (0.67)	0.704	0.024 (0.01)	5.36 (2.24)	1.89 (2.22)*	0.729	0.025 (0.008)	3.53 (0.43)	0.732
Duke Pine	0.057 (0.019)	7.14 (1.36)	0.170	0.007 (0.02)*	10.96 (2.67)	1.15 (0.58)	0.522	0.0006 (0.013)*	6.03 (0.49)	0.529
Alpine Ash	0.016 (0.016)*	11.98 (1.0)	0.716	0.001 (0.01)*	14.43 (2.37)	0.95 (0.33)	0.801	0.0001 (0.014)*	6.9 (0.45)	0.798
Macchia	0.038 (0.008)*	9.09 (0.92)	0.684	0.03 (0.01)	14.7 (10.1)*	1.22 (1.25)*	0.631	0.029 (0.01)	7.55 (0.87)	0.623
Fagus	-0.002 (0.015)*	11.24 (0.88)	0.881	-0.06 (0.04)*	8.17 (1.94)	7.28 (10.0)*	0.782	-0.036 (0.025)*	8.13 (0.94)	0.772
Savanna	0.048 (0.015)	13.62 (0.89)	0.756	0.023 (0.02)*	141.3 (524)*	0.125 (0.5)*	0.77	0.0013 (0.02)*	12.49 (0.95)	0.698
Red Gum	0.016 (0.007)	15.27 (1.03)	0.702	0.014 (0.007)	68.7 (103)*	0.43 (0.72)*	0.739	0.01 (0.007)*	14 (0.94)	0.703