



THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

How do leaf and ecosystem measures of water-use efficiency compare?

Citation for published version:

Medlyn, BE, De Kauwe, MG, Lin, YS, Knauer, J, Duursma, RA, Williams, CA, Arneeth, A, Clement, R, Isaac, P, Limousin, JM, Linderson, ML, Meir, P, Martin-StPaul, NK & Wingate, L 2017, 'How do leaf and ecosystem measures of water-use efficiency compare?' *New Phytologist*. DOI: 10.1111/nph.14626

Digital Object Identifier (DOI):

[10.1111/nph.14626](https://doi.org/10.1111/nph.14626)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Peer reviewed version

Published In:

New Phytologist

General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.



1 **How do leaf and ecosystem measures of water-use efficiency compare?**

2

3 Belinda E. Medlyn¹, Martin G. De Kauwe², Yan-Shih Lin^{2,3}, Jürgen Knauer^{1,4}, Remko A.
4 Duursma¹, Christopher A. Williams^{1,5}, Almut Arneth⁶, Rob Clement⁷, Peter Isaac⁸, Jean-
5 Marc Limousin⁹, Maj-Lena Linderson¹⁰, Patrick Meir^{7,11}, Nicolas Martin-StPaul¹², Lisa
6 Wingate¹³

7

8

- 9 1. Hawkesbury Institute for the Environment, Western Sydney University, Locked Bag
10 1797, Penrith NSW 2751 Australia
- 11 2. Dept of Biological Science, Macquarie University, North Ryde NSW 2109 Australia
- 12 3. Ecologie et Ecophysiologie Forestières, Centre INRA de Nancy-Lorraine, Route
13 d'Amance, 54280 Champenoux France
- 14 4. Department of Biogeochemical Integration, Max Planck Institute for
15 Biogeochemistry, 07745 Jena, Germany
- 16 5. Graduate School of Geography, Clark University, 950 Main Street, Worcester, MA
17 01602 USA
- 18 6. Karlsruhe Institute of Technology, Dept. Atmospheric Environmental Research
19 (IMK-IFU), Kreuzackbahnstr. 19, 82467 Garmisch-Partenkirchen
- 20 7. School of Geosciences, University of Edinburgh, Edinburgh EH93FF, UK
- 21 8. OzFlux, Melbourne, Australia
- 22 9. Centre d'Ecologie Fonctionnelle et Evolutive CEFE, UMR 5175, CNRS - Université
23 de Montpellier - Université Paul-Valéry Montpellier – EPHE, 1919 Route de Mende,
24 34293 Montpellier Cedex 5, France
- 25 10. Dept. of Physical Geography and Ecosystem Science, Lund University, Sölvegatan
26 12, SE-262 33 Lund, Sweden
- 27 11. Research School of Biology, Australian National University, Canberra ACT 2601,
28 Australia
- 29 12. INRA, UR629 Ecologie des Forêts Méditerranéennes (URFM), Avignon, France
- 30 13. ISPA, Bordeaux Sciences Agro, INRA, 33140, Villenave d'Ornon, France

31

32 **For submission to: NewPhytologist**

33

34

35 **No of words in Abstract: 198**

36

37 **No of words in Main text: 5190**

38

39 **No of figures: 7**

40

41 **No of tables: 2**

42

43

44 **Abstract**

45

46 The terrestrial carbon and water cycles are intimately linked: the carbon cycle is driven by
47 photosynthesis, while the water balance is dominated by transpiration, and both fluxes are
48 controlled by plant stomatal conductance. The ratio between these fluxes, the plant water
49 use efficiency (WUE), is a useful indicator of vegetation function. WUE can be estimated
50 using several techniques, including leaf gas exchange, stable isotope discrimination, and
51 eddy covariance. Here we compare global compilations of data for each of these three
52 techniques. We show that patterns of variation in WUE across plant functional types are
53 not consistent among the three datasets. Key discrepancies include: (1) Leaf-scale data
54 indicate differences between needle-leaf and broadleaf forests, but ecosystem-scale data
55 do not; (2) leaf-scale data indicate differences between C3 and C4 species, whereas at
56 ecosystem scale there is a difference between C3 and C4 crops but not grasslands; and (3)
57 isotope-based estimates of WUE are higher than estimates based on gas exchange for most
58 PFTs. Our study quantifies the uncertainty associated with different methods of measuring
59 WUE, indicates potential for bias when using WUE measures to parameterise or validate
60 models, and indicates key research directions needed to reconcile alternative measures of
61 WUE.

62

63 **Keywords:** stomatal conductance, water use efficiency, plant functional type, stable
64 isotopes, leaf gas exchange, eddy covariance

65

66

67 **Introduction**

68

69 One of the fundamental trade-offs governing plant growth is the exchange of water for
70 carbon: land plants must open their stomata to take up carbon dioxide in order to grow,
71 but at the same time water vapour is lost via transpiration, with the concomitant risk of
72 desiccation (Cowan & Farquhar 1977). This trade-off can be characterised by the plant's
73 water-use efficiency (WUE), defined as the amount of carbon taken up per unit water
74 used (Sinclair et al. 1984). Combining as it does the key processes of photosynthesis and
75 transpiration, WUE is a widely-used parameter indicating vegetation performance.

76

77 Water-use efficiency can be estimated using several methods that operate at different
78 temporal and spatial scales. Community research efforts have led to the compilation of
79 global datasets based on each of these methods. These datasets are increasingly being
80 utilised to constrain and evaluate global vegetation models (e.g. Groenendijk et al. 2011,
81 Saurer et al. 2014, Kala et al. 2015, Dekker et al. 2016). However, there has to date been
82 little comparison across methods. It is often assumed that values obtained at one scale
83 should be relatable to values obtained at other scales, but this assumption has not been
84 explicitly tested across ecosystems. Our goal in this paper is to compare three
85 independent global datasets of WUE, obtained using leaf gas exchange, stable isotope,
86 and eddy covariance techniques, and investigate whether global patterns obtained using
87 these different techniques are consistent with our current understanding of scaling.
88 Specifically, we focus on patterns of variation across plant functional types (PFTs),
89 which are used to represent vegetation in global vegetation models, and ask whether the
90 three datasets indicate consistent differences among PFTs.

91

92 WUE is known to vary with atmospheric vapour pressure deficit (VPD) (Monteith 1986).
93 To compare across datasets, a metric of WUE is required that accounts for this variation.
94 One commonly-used metric is the intrinsic WUE (iWUE), defined as photosynthetic C
95 uptake divided by stomatal conductance to water vapour (A/g_s). Another related metric is
96 the ratio of intercellular to atmospheric CO₂ (C_i/C_a ratio). However, both iWUE and the
97 C_i/C_a ratio also vary with VPD, meaning that values obtained under different VPD

98 conditions cannot be directly compared. In this work, we account for variation in VPD
99 conditions by using the parameter, g_1 , of a recent model of stomatal conductance (g_s mol
100 $m^{-2} s^{-1}$), derived from the theory of optimal stomatal behaviour (Medlyn et al. 2011):
101

$$g_s = 1.6 \left(1 + \frac{g_1}{\sqrt{D}} \right) \frac{A}{C_s} \quad (1)$$

102
103 where A is the net assimilation rate ($\mu\text{mol } m^{-2} s^{-1}$), C_s ($\mu\text{mol } \text{mol}^{-1}$) and D (kPa) are the
104 CO_2 concentration and the vapour pressure deficit at the leaf surface, respectively. The
105 model parameter g_1 ($\text{kPa}^{0.5}$) represents normalised plant water use efficiency. The model
106 parameter g_1 is inversely related to iWUE but accounts for VPD, by assuming a \sqrt{D}
107 dependence of the C_i/C_a ratio, as found for leaf gas exchange (Medlyn et al. 2011) and
108 eddy covariance data (Zhou et al. 2015). ~~and can thus be directly compared across~~
109 ~~datasets.~~ This parameter also corrects for increases in WUE driven by changes in C_a . If
110 the ratio C_i/C_a is constant with increasing C_a , then g_1 is also constant (Medlyn et al.
111 2011). Assuming that these relationships accurately account for environmental effects on
112 WUE, the parameter g_1 is then a measure of WUE that can ~~and can thus be directly~~
113 compared across datasets.

114
115 We apply this model to three major global data compilations. Lin et al. (2015) compiled a
116 global database of leaf gas exchange measurements, including photosynthetic rate and
117 stomatal conductance, and used these data to estimate instantaneous values of g_1 . Lin et
118 al. (2015) found systematic differences in g_1 among PFTs, with high values of g_1 (and
119 thus low iWUE) in crops, C3 grasses and deciduous angiosperm trees, and low values in
120 C4 grasses and gymnosperms. Leaf-level gas exchange data such as these are commonly
121 used to parameterise stomatal behaviour in vegetation models (e.g. Bonan et al. 2014).
122 The differences among PFTs observed by Lin et al. (2015) have important consequences
123 for modelled vegetation function at large scales, including changes in predicted surface
124 cooling and consequent heatwave development (Kala et al. 2015, 2016).
125

126 Stable isotope methods can be applied to plant tissue to estimate $iWUE$ and g_1 values
127 over monthly to annual time scales (Farquhar et al. 1989, Cernusak et al. 2013). Long-
128 term stable isotope records from tree rings are widely used to constrain model predictions
129 of WUE at large spatial and temporal scales (e.g. Frank et al. 2015, Saurer et al. 2015,
130 Dekker et al. 2016). A compilation of leaf ^{13}C discrimination measurements indicated
131 differences in stomatal behaviour among PFTs (Diefendorf et al. 2010). Here, we
132 estimated g_1 values from a global database of nearly 4,000 measurements of bulk leaf ^{13}C
133 discrimination ($\Delta^{13}C$), taken from 594 sites spread across all seven continents (Cornwell
134 et al, in review). We predicted that values of g_1 estimated from this dataset would show
135 similar rankings across PFTs as the leaf gas exchange data set, but that values would be
136 lower, due to mesophyll resistance to CO_2 diffusion (Seibt et al. 2008).

137
138 At larger spatial scales, eddy flux measurements can be used to estimate whole-
139 ecosystem gross primary productivity (GPP) and evapotranspiration (ET), and their ratio
140 GPP/ET , which is the whole-ecosystem water-use efficiency (Law et al. 2002, Beer et al.
141 2009, Keenan et al. 2013). These data are also being widely applied to constrain and
142 evaluate vegetation models (e.g. Groenendijk et al. 2011, Bonan et al. 2012, Haverd et al.
143 2013). We predicted that g_1 values estimated from these data would show similar
144 rankings across PFTs as the leaf gas exchange and stable isotope datasets, but that
145 estimated values of g_1 would be higher due to the contribution of non-transpiratory water
146 vapour fluxes to evapotranspiration (i.e. free evaporation from soil and canopy).

147

148

149 **Methods**

150

151 *Datasets*

152 We synthesised three independent datasets to estimate values of g_1 . All datasets, and our
153 analysis code, are available online; web addresses are given below under “data deposition
154 statement”. Leaf gas exchange data were taken from Lin et al. (2015), who collated
155 measurements under ambient field conditions from 286 species, covering 56 sites across
156 the globe. The majority of these data are measurements on upper-canopy leaves during

157 the growing season. Isotope data came from a global database of leaf carbon isotopes
158 measurements from natural and semi-natural habitats, across 3985 species-sites
159 combinations (Cornwell et al. in review). Flux measurements were taken from the global
160 collection of eddy flux measurements that comprise the FLUXNET “La Thuile” Free and
161 Fair dataset (<http://www.fluxdata.org>). This dataset contains gap-filled, half-hourly
162 measurements of carbon dioxide, water vapour and energy fluxes; following filtering (see
163 below) we were able to use data from 120 sites. The global distribution of the three
164 datasets is shown in Figure 1.

165

166 *Estimating g_1*

167 g_1 was estimated from leaf gas exchange data using non-linear regression to fit the
168 unified stomatal optimisation model (Medlyn et al. 2011, eqn 1) to g_s measurements for
169 each species. Here we followed the methods of Lin et al. (2015). All model fits were
170 done using the “minimize” function of the python “lmfit” library, using the Levenberg-
171 Marquardt method (Newville et al. 2014).

172

173 Cornwell et al. (in review) estimated carbon isotope discrimination (Δ) values from bulk
174 leaf $\delta^{13}\text{C}$ and estimates of source air $\delta^{13}\text{C}$ composition. From these data, we estimated
175 the ratio of the intercellular to ambient carbon dioxide concentration (C_i/C_a) following
176 Farquhar et al. (1989) for C_3 species:

177

$$\frac{C_i}{C_a} = \frac{\Delta - a}{b - a} \quad (2)$$

178

179 where a represents the fractionation caused by gaseous diffusion (4.4‰) and b is the
180 effective fractionation caused by carboxylating enzymes (assumed to be 27‰) (Cernusak
181 et al. 2013). Note that we were unable to utilise values for C4 vegetation from this
182 dataset. For C4 plants, the relationship between C_i/C_a and $\Delta^{13}\text{C}$ depends on bundle sheath
183 leakiness, ϕ (Henderson et al. 1998; Cernusak et al. 2013). Adopting a value for ϕ of 0.21

184 for C4 vegetation, as suggested by Henderson et al. (1998), yielded unrealistic estimates
185 of $C_i/C_a < 0$ for more than half (79/140) of the dataset.

186

187 Values of g_1 for C3 species were estimated following Medlyn et al. (2011):

188

$$g_1 = \frac{\left(\frac{C_i}{C_a} \sqrt{D}\right)}{\left(1 - \frac{C_i}{C_a}\right)} \quad (3)$$

189

190 Mean daytime growing season VPD was estimated from monthly mean and maximum
191 temperature and relative humidity data obtained from the Climatic Research Unit (CRU
192 1.0) 0.5-degree gridded monthly climatology (New et al. 2002). Growing season was
193 defined as the time period during which the daytime mean temperature is above zero. All
194 values were estimated on a monthly basis then linearly interpolated to a daily basis. Daily
195 VPD estimates could then be averaged over the growing season.

196

197 Values of g_1 were estimated from FLUXNET data as follows. First, canopy stomatal
198 conductance (G_s) was estimated from LE flux ($\text{J m}^{-2} \text{s}^{-1}$) as

199

$$G_s = \frac{LE/\lambda}{D/P} \quad (4)$$

200

201 where λ is the latent heat of water vapour (J mol^{-1}), D (Pa) is the vapour pressure deficit
202 and P is the atmospheric pressure (Pa). Pressure was estimated using the hypsometric
203 equation based on site elevation data. Where site elevation information was missing,
204 values were gap-filled using the 30-arc seconds (~ 1 km) global digital elevation model
205 GTOPO30 data from the United States Geological Survey (USGS). Values of g_1 were
206 then estimated by fitting eqn (1) to data, taking G_s for g_s and gross primary productivity
207 (GPP) for A .

208

209
210 FLUXNET data were screened as follows: (i) data flagged as “good”; (ii) data from the
211 three most productive months, in terms of flux-derived GPP (to account for the different
212 timing of summer in the Northern and Southern hemispheres); (iii) daylight hours

213 between 9 am and 3 pm; (iv) time slices with precipitation, as well as the subsequent 48
 214 half-hour time slices, were excluded (to minimise contributions from soil/wet canopy
 215 evaporation); (v) time slices with missing CO₂ data were gap-filled with the global
 216 annual mean from averaged marine surface (<http://www.esrl.noaa.gov/gmd/ccgg/trends/>).
 217 If the entire year's data were missing, or if the annual mean departed from the global
 218 mean by $\pm 15\%$, data were replaced with the global mean. This screening check was used
 219 to address possible errors in locally recorded CO₂ concentrations in 14 site-year
 220 combinations, which showed drops against a global trend of increasing CO₂
 221 concentrations (1995–2004: 1.87 ppm yr⁻¹). In addition, fitted g_1 values with an $R^2 < 0.2$
 222 were excluded, as were fitted g_1 values that were $\pm 50\%$ from the site average.

223
 224 We used equation (4) to estimate canopy conductance as this approach is taken in a
 225 number of other studies (e.g. Beer et al. 2009, Keenan et al. 2013) and the equation can
 226 be applied to all Fluxnet datasets. However, the use of equation (4) to estimate canopy
 227 conductance is a simplification because it assumes that the vegetation is fully coupled to
 228 the surrounding atmosphere, and therefore that water vapour exchange is directly
 229 proportional to stomatal conductance. There is also an aerodynamic resistance to gas
 230 exchange, resulting in a partial decoupling of canopy-atmospheric gas exchange,
 231 particularly in short-statured vegetation (Jarvis & McNaughton 1986). To estimate values
 232 of g_1 accounting for aerodynamic resistance, G_s was estimated by inverting the Penman-
 233 Monteith equation from measured LE flux:

234

$$G_s = \frac{G_a \gamma \lambda E}{s(R_n - G) - (s + \gamma)\lambda E + G_a M_a c_p D} \quad (5)$$

235

236 where G_a (mol m⁻² s⁻¹) is the canopy aerodynamic conductance, λ is the latent heat of
 237 water vapour (J mol⁻¹), E (mol m⁻² s⁻¹) is the canopy transpiration, γ is the psychrometric
 238 constant (Pa K⁻¹), s is the slope of the saturation vapour pressure curve at air temperature
 239 (Pa K⁻¹), R_n (W m⁻²) is the net radiation, D (Pa) is the vapour pressure deficit, G (W m⁻²)
 240 is the soil heat flux, M_a (kg mol⁻¹) is molar mass of air, c_p is the heat capacity of air (J kg⁻¹
 241 K⁻¹). At sites where values of G were not available, G was set to zero. G_a was calculated

242 as $P / (R_{\text{gas}} T_k) / (u/u^* + 6.2u^{*-2/3})$ where u^* (m s^{-1}) is friction velocity and u (m s^{-1}) is wind
243 speed (Thom et al. 1972). P is atmospheric pressure (Pa), R_{gas} is the gas constant (J mol^{-1}
244 K^{-1}), T_k is the air temperature in Kelvin, and the term $P / (R_{\text{gas}} T_k)$ converts from units of
245 m s^{-1} to $\text{mol m}^{-2} \text{s}^{-1}$. Equation (5) was applied to all datasets where R_n and u^* were
246 available. Inspection of equation (5) shows that, under most conditions, incorporating a
247 finite G_a value will lead to a lower estimate of G_c than would be obtained with infinite
248 G_a .

249

250 *Ancillary data*

251 The isotope dataset does not contain information on PFTs; these were determined from
252 species information online. If we were unable to assign a PFT, data were excluded from
253 further analysis. For Fluxnet data, the PFTs WSA (woody savannah) and SAV
254 (savannah) were combined into SAV, and PFTs OSH (open shrublands) and CSH (closed
255 shrublands) were combined into SHB. PFT MF (mixed forest) was omitted. Data
256 screening led to a loss of 12% from the isotope dataset and ~35% from the FLUXNET
257 dataset.

258

259 Estimates of the relative fraction of C4 present at each FLUXNET site were derived from
260 the closest matching 0.5-degree pixel in the North American Carbon Program (NACP)
261 Global C3 and C4 SYnergetic land cover MAP (SYNMAP) (Jung et al. 2006).

262

263 Peak LAI for FLUXNET sites was obtained from the site-level ancillary data when
264 available in the supporting documents contributed to the La Thuile Synthesis Collection
265 (see www.fluxdata.org).

266

267 *Statistics*

268 We tested for statistical differences among methods by applying one-way ANOVA to
269 log-transformed values of $g_{1\text{-leaf}}$, $g_{1\text{-isotope}}$ and site-averaged $g_{1\text{-flux}}$ for each PFT. For each
270 method, we used a mixed-model approach to test for differences among PFTs, taking site
271 as a random factor. Similarly, a mixed-model approach was used to test for statistical

272 differences among PFTs for a given method. Differences among methods and among
273 PFTs were identified using Tukey's Honest Significant Difference.
274
275

276 **Results**

277

278 Values of g_1 estimated using the three alternative methods differed significantly within
279 most PFTs (Figure 2). In addition, the variation in g_1 across PFTs was not consistent
280 among the three methods (Table 1).

281

282 *Forest PFTs*

283 Among the four forest PFTs, median values of g_1 derived from leaf gas exchange ($g_{1\text{-leaf}}$)
284 were lowest in evergreen needleleaf forest (ENF), intermediate in evergreen broadleaf
285 forest (EBF) and highest in deciduous broadleaf forest (DBF) and tropical rainforest
286 (TRF). Isotope-derived values of g_1 ($g_{1\text{-isotope}}$) mostly had similar variation across forest
287 types as $g_{1\text{-leaf}}$ values: they were lowest in ENF, intermediate in EBF and DBF, but were
288 significantly larger in TRF. In clear contrast to other two datasets, there were no
289 significant differences among forest types for values of g_1 derived from flux data ($g_{1\text{-flux}}$).
290 Values of $g_{1\text{-flux}}$ for ENF and EBF were higher than those of the other datasets.

291

292 Values of $g_{1\text{-isotope}}$ were generally lower than values of $g_{1\text{-leaf}}$ for a given PFT, with the
293 exception of TRF (Figure 2). The largest difference between $g_{1\text{-leaf}}$ and $g_{1\text{-isotope}}$ was
294 observed for DBF species, whereas there was no significant difference in mean values for
295 EBF and TRF species. For the TRF PFT, $g_{1\text{-isotope}}$ values were often unrealistically high;
296 inferred values of $C_i/C_a > 0.95$ resulted in values of $g_{1\text{-isotope}} > 20 \text{ kPa}^{0.5}$. Such high
297 values were not limited to one dataset, but were observed in a number of TRF datasets.

298

299 *Non-forest PFTs*

300 Among the non-forest PFTs, $g_{1\text{-leaf}}$ values were significantly higher in C3 grasses (C3G)
301 than C4 grasses (C4G), intermediate in shrubs (SHB), and rather variable in savannah
302 (SAV) trees. The variability of $g_{1\text{-leaf}}$ in SAV is likely related to the high seasonality in
303 these systems: this instantaneous measure of WUE can vary considerably between wet
304 and dry seasons. Note that the comparison among methods for the SAV PFT is somewhat
305 biased because eddy covariance data are from the whole ecosystem and thus include both
306 trees and understorey, whereas leaf gas exchange for this PFT is from trees only while

307 isotope data are principally from trees and shrubs. As with forest PFTs, values of $g_{1\text{-isotope}}$
308 for non-forest PFTs were on average lower than values of $g_{1\text{-leaf}}$, but the rankings of PFTs
309 differed: C₃ grasses had lower $g_{1\text{-isotope}}$ values than SAV or SHB, an unexpected result.
310 We were unable to estimate values of $g_{1\text{-isotope}}$ for C₄ species (see Methods) although
311 $\Delta^{13}\text{C}$ values clearly differed between C₃ and C₄ vegetation (Cornwell et al. in review).

312

313 Photosynthetic pathway had a significant effect on $g_{1\text{-flux}}$ values for crop vegetation: $g_{1\text{-flux}}$
314 was significantly lower in C₄ crops (C₄C) than C₃ crops (C₃C). Values of $g_{1\text{-flux}}$ were
315 high for grasslands (C₃G), similar to $g_{1\text{-leaf}}$ values and much higher than $g_{1\text{-isotope}}$ values.
316 We did not find evidence that the presence of C₄ grasses reduced $g_{1\text{-flux}}$ in grasslands
317 (Figure 3); grassland $g_{1\text{-flux}}$ values were not correlated with estimated C₄ fraction.

318

319 *Comparison of forest and non-forest PFTs*

320 Apart from C₄C, median values of $g_{1\text{-flux}}$ were somewhat higher for non-forest than forest
321 vegetation, and were particularly high for SHB. It is possible that the contribution of soil
322 evaporative flux to total evapotranspiration is higher in these more open systems,
323 resulting in larger $g_{1\text{-flux}}$ values. This conclusion is supported by an examination of the
324 influence of leaf area index (LAI) on $g_{1\text{-flux}}$ for forest and non-forest vegetation, for sites
325 where LAI estimates were available (Figure 4). At lower LAI (up to 3 m² m⁻²), values of
326 $g_{1\text{-flux}}$ were more variable for non-forest than forest sites, with several non-forest sites
327 showing values of $g_{1\text{-flux}}$ greater than 8 kPa^{0.5}, providing some support for the inference
328 that soil evaporative fluxes play a larger role in non-forest ecosystems.

329

330 *Exploration of inconsistent patterns among datasets*

331 The lack of difference among $g_{1\text{-flux}}$ values for forest PFTs was unexpected. The
332 consistent evidence from $g_{1\text{-leaf}}$ and $g_{1\text{-isotope}}$ values suggests that leaf-scale g_1 is low for
333 ENF. We had anticipated that this difference would scale to canopy behaviour, yet there
334 is no evidence that $g_{1\text{-flux}}$ values were lower for this PFT. It is possible that sampling
335 biases lead to different results for the three methodologies. To investigate this possibility,
336 we first compared the latitudinal distributions of the three datasets, using latitude as an
337 indicator of climatic conditions (Figure 5). Clear differences in sampling coverage with

338 latitude can be seen. However, Figure 5 demonstrates that irrespective of latitude, values
339 of $g_{1\text{-leaf}}$ and $g_{1\text{-isotope}}$ are lower in ENF than in DBF, whereas values of $g_{1\text{-flux}}$ are similar
340 between ENF and DBF.

341

342 To further rule out sampling bias, we also compared half-hourly leaf gas exchange data
343 and eddy flux data for eight sites where both kinds of data were available (Figure 6). This
344 direct comparison shows that $g_{1\text{-leaf}}$ and $g_{1\text{-flux}}$ values were in a similar range for DBF and
345 TRF forest types but that $g_{1\text{-leaf}}$ was lower than $g_{1\text{-flux}}$ for EBF and ENF forest types,
346 further confirming that the discrepancy between $g_{1\text{-leaf}}$ and $g_{1\text{-flux}}$ is not simply a result of
347 sampling bias.

348

349 We tested whether decoupling of canopy-atmosphere gas exchange could explain the
350 discrepancy between the cross-PFT patterns in $g_{1\text{-leaf}}$ and $g_{1\text{-flux}}$ values. We estimated
351 canopy stomatal conductance from eddy flux data using the Penman-Monteith (PM)
352 equation (equation 5), which incorporates an aerodynamic resistance term. Applying the
353 PM equation results in a large reduction in estimated values of $g_{1\text{-flux}}$ for all PFTs (Figure
354 7). For PFTs where $g_{1\text{-flux}}$ previously exceeded $g_{1\text{-leaf}}$, the values become comparable (e.g.
355 ENF). However, for PFTs where $g_{1\text{-flux}}$ was previously comparable with $g_{1\text{-leaf}}$, the values
356 become significantly lower (e.g. DBF, C3G). Thus, consideration of decoupling does not
357 resolve the inconsistency in cross-PFT patterns between $g_{1\text{-leaf}}$ and $g_{1\text{-flux}}$.

358

359 **Discussion**

360

361 Our comparison of g_1 values across three global datasets provides a number of new
362 insights into patterns of water use efficiency across scales, and highlights some important
363 inconsistencies in the datasets. The parameter g_1 is inversely related to water-use
364 efficiency, such that plants with high WUE have low g_1 and vice-versa. We had predicted
365 that g_1 values would vary consistently across PFTs in all three datasets, but our results did
366 not support this prediction, as there were significantly different patterns across PFTs in
367 each dataset. We also predicted that g_1 values would vary across methods, with the lowest
368 values obtained from isotope data, and the highest values obtained from flux data. The

369 first part of this prediction was largely supported, with lower $g_{1\text{-isotope}}$ than $g_{1\text{-leaf}}$ for most
370 PFTs, but the second part of the prediction was not, as $g_{1\text{-flux}}$ values were not in general
371 higher than $g_{1\text{-leaf}}$, particularly when decoupling between the canopy and atmosphere was
372 taken into account.

373

374 *Cross-PFT patterns compared among datasets*

375 For forest vegetation, there was an important discrepancy in cross-PFT patterns between
376 leaf and ecosystem-scale estimates of g_1 . At leaf scale, a difference between needle-leaf
377 (ENF) and deciduous broad-leaf (DBF) forests is seen in both leaf gas exchange and
378 stable isotope data, as has also been found in previous studies (e.g. Lloyd & Farquhar
379 1994, Diefendorf et al. 2010). Our current understanding of scaling between leaves and
380 ecosystems suggests that a similar difference between these PFTs should be seen in g_1
381 estimated from eddy covariance data. Intriguingly, however, no such difference was
382 observed; values of $g_{1\text{-flux}}$ were similar for all forest PFTs (Figures 2, 6). This
383 inconsistency between datasets has important consequences for our ability to model water
384 use efficiency at larger scales, since it implies that models parameterised with leaf gas
385 exchange or stable isotope data will not agree with flux data, or with models
386 parameterised using flux data.

387

388 Consideration of decoupling between stomata and atmosphere (*sensu* Jarvis &
389 McNaughton 1986) did not help to explain this discrepancy (Figure 7). We found that
390 there was no difference in $g_{1\text{-flux}}$ among forest types irrespective of whether the estimation
391 of $g_{1\text{-flux}}$ incorporated a decoupling factor. We found that median $g_{1\text{-flux}}$ approached
392 median $g_{1\text{-leaf}}$ for needle-leaf forests when decoupling was considered, and for broadleaf
393 forests when it was not. This observation is supported by previous studies of scaling on
394 single forests: a study on water-use efficiency in Scots pine found congruence between
395 leaf and canopy water-use efficiency using a scaling approach incorporating decoupling
396 (Launiainen et al. 2011) whereas studies in broadleaf forests find congruence using
397 approaches that do not consider decoupling (Linderson et al. 2012, Barton et al. 2012).
398 However, it is generally thought that decoupling should be smallest in needleleaf
399 canopies (Jarvis & McNaughton 1986). This discrepancy clearly requires further

400 investigation. [Refining estimates of canopy stomatal and non-stomatal conductances from](#)
401 [eddy flux data is one potential way forwards \(e.g. Wehr et al. 2017\).](#)

402

403 Leaf gas exchange also indicates a large difference in g_1 between C₃ and C₄ species, as
404 expected from their physiology. Although there was a clear difference in $\Delta^{13}\text{C}$ between
405 these two groups of species, we were unable to estimate $g_{1\text{-isotope}}$ for the C₄ species and
406 hence unable to substantiate this difference in g_1 at leaf level using isotopic data. The
407 issues involved in estimating C_i/C_a from $\Delta^{13}\text{C}$ in C₄ plants are discussed by Cernusak et
408 al. (2013). A simple linear relationship was proposed by Henderson et al. (1992) but
409 requires an estimate of bundle-sheath leakiness, ϕ . Cernusak et al. (2013) suggest that $\phi <$
410 0.37 under most environmental conditions. With this value of ϕ , the linear relationship
411 yields unrealistic values of C_i/C_a for much of the dataset, as the majority of measured
412 values have $\Delta^{13}\text{C} > 4.4\text{‰}$. These data imply that either a value for $\phi > 0.37$ is more
413 commonly found in field conditions, or else that the simple linear relationship between
414 $\Delta^{13}\text{C}$ and C_i/C_a is inaccurate for leaf dry matter. Further research is needed to establish
415 more widely-applicable relationships between stable isotope data and water-use
416 efficiency for C₄ species.

417

418 Nonetheless, a difference in leaf-level g_1 between C₃ and C₄ species is well-documented
419 in the literature (e.g. Morison & Gifford 1983; Ghannoum et al. 2010). Earlier studies
420 synthesising water-use efficiency from eddy covariance data did not explicitly address
421 photosynthetic pathway (Law et al. 2002, Beer et al. 2009), and thus it was not known
422 whether this fundamental leaf-level difference in g_1 is reflected in canopy scale gas
423 exchange. Zhou et al. (2016) reported a difference in “underlying WUE”, an index
424 similar to g_1 , between C₃ (corn) and C₄ (soybean) crops at 5 Ameriflux sites. Similarly,
425 we found a significant difference in $g_{1\text{-flux}}$ between C₃ and C₄ crops that is consistent
426 with the difference in $g_{1\text{-leaf}}$ (Figure 2). However, we did not find any evidence for lower
427 $g_{1\text{-flux}}$ for grasslands with a C₄ component (Figure 3). The difference in $g_{1\text{-flux}}$ between C₃
428 and C₄ crops demonstrates that differences in $g_{1\text{-leaf}}$ can scale to whole canopies, and that
429 photosynthetic pathway must be considered when interpreting fluxes from crop canopies.
430 The lack of an influence of photosynthetic pathway on grassland $g_{1\text{-flux}}$, in contrast to

431 crops, has several potential explanations. It is possible that there are significant
432 evaporative fluxes from soil in grasslands that compensate for differences in transpiration
433 between C3 and C4 vegetation. However, we also note that, owing to a lack of
434 information at site scale, we were obliged to estimate C4 fraction in grasslands from a
435 global dataset with relatively coarse resolution, suggesting that our characterisation of C4
436 fraction may have been inaccurate. To correctly interpret fluxes from grasslands with a
437 significant C4 component requires better quantification of vegetation C3/C4 fraction at
438 the site level. Furthermore, the estimated grassland C4 fraction did not exceed 0.4; data
439 from grasslands known to have high C4 fraction is needed to test robustly for this effect.
440 Finally, there is very high variability across site-years in g_1 -flux estimates for C3-only
441 grasslands (Figure 3), meaning our test lacks power; a better understanding of the reasons
442 for this variability is needed to design fairer comparisons between C3 and C4-dominated
443 grasslands.

444

445 *Relative g_1 values from different methods*

446 We predicted that $g_{1\text{-flux}}$ values would exceed $g_{1\text{-leaf}}$ values, due to additional water vapour
447 loss from soil or canopy evaporation (cf. Figure 4). In contrast to our prediction, we
448 found that once decoupling was taken into account, values of median $g_{1\text{-flux}}$ were lower
449 than values of $g_{1\text{-leaf}}$ for several PFTs (Figure 7). Significant within-canopy gradients in
450 $g_{1\text{-leaf}}$ can occur (e.g. Company et al. 2017), but consideration of these gradients would
451 also result in larger $g_{1\text{-flux}}$ than canopy-top $g_{1\text{-leaf}}$. One potential explanation may be
452 related to the use of GPP in the calculation of $g_{1\text{-flux}}$, rather than net photosynthesis (i.e.
453 gross photosynthesis, less leaf respiration) as is used in the calculation of $g_{1\text{-leaf}}$. Recent
454 work by Wehr et al. (2016) also suggests that the current method used to estimate GPP
455 can over-estimate daytime foliar respiration, which would tend to exaggerate the
456 difference between GPP and net canopy photosynthesis. Further research is required to
457 quantify the effect of including foliage respiration in estimation of $g_{1\text{-flux}}$, to determine if
458 this mechanism is sufficient to account for low $g_{1\text{-flux}}$ values.

459

460 We also predicted that $g_{1\text{-isotope}}$ values would be ~~somewhat~~ lower than those of $g_{1\text{-leaf}}$ due
461 to mesophyll conductance (g_m), which is neglected in the simplified isotopic theory used

462 here to relate leaf isotopic composition to C_i/C_a ratio (Seibt et al. 2008)–(Evans et al.
463 1986), though it has been suggested that the value of b used here (Eq. 2) should at least
464 partially account for g_m effects (Seibt et al. 2008, Cernusak et al. 2013). In support of
465 ~~this our~~ prediction, median values of $g_{1\text{-isotope}}$ were lower than median values of $g_{1\text{-leaf}}$ for
466 all PFTs other than tropical rainforest (Figure 2). The size of this effect should increase
467 with increasing drawdown of CO_2 from the intercellular airspace to the site of
468 carboxylation; this drawdown is high in plants with low mesophyll conductance
469 (typically ENF and EBF species, Niinemets et al. 2009) and/or high photosynthetic rates.
470 Nonetheless, we were surprised by the magnitude of the difference, which was substantial
471 in most PFTs. Previous smaller-scale studies have found a good correspondence between
472 leaf isotope and gas exchange measurements of C_i/C_a (e.g. Farquhar et al. 1982, Orchard
473 et al. 2010). The size of this difference in our global data comparison suggests that use of
474 the values of $g_{1\text{-isotope}}$ to constrain large-scale models requires that g_m be taken into
475 account. To do so, models will need a general quantitative knowledge of the drawdown
476 of CO_2 from the intercellular space to the mesophyll, which depends on both g_m and the
477 photosynthetic rate (Evans et al. 1986). As woody tissue is generally ^{13}C enriched
478 compared to leaf tissue (Cernusak et al. 2009), values of g_1 estimated from tree ring
479 stable isotopes would likely be lower still.

480
481 One exception to this general pattern of lower $g_{1\text{-isotope}}$ values was the TRF PFT (Figure
482 2). Very high $g_{1\text{-isotope}}$ values were obtained for tropical rainforest species by comparison
483 with other PFTs. These high values may indicate that the leaves used for these
484 measurements were exposed to air with a signature of recent respiration and a
485 correspondingly low ^{13}C fraction, although previous studies suggest that this effect
486 should only be important in the lower canopy (Buchmann et al. 2002). A further potential
487 explanation is that our estimates of long-term average daytime VPD, taken from a global
488 climate dataset (see Methods), do not reflect in-canopy VPD values experienced by
489 sampled leaves, particularly in high-humidity conditions typical of the TRF PFT.

490
491 *Dataset biases*

492 Each of the three datasets used in this study represents an enormous global scientific
493 effort, and each is extremely valuable in advancing our understanding of the role of
494 terrestrial vegetation in global carbon and water cycles. Nonetheless, each approach is
495 subject to limitations. Leaf gas exchange measures are a direct and relatively accurate
496 measure of the performance of a single leaf at a given point in time, but are inevitably
497 restricted in sampling coverage. Measurements are often made only at the top of the
498 canopy, for example, or only on a few days per season. There are some more extensive
499 datasets in the Lin et al. (2015) database that were gathered through the use of in-situ
500 cuvettes (e.g. Kolari et al. 2007, Op de Beeck et al. 2010, Tarvainen et al. 2013), but
501 these remain the exception rather than the rule, and in any case cannot capture all
502 potential sources of variation in the canopy. Stable isotope measures are more extensive
503 (Figure 2) but are less direct measures of gas exchange, and, as our results show, may be
504 influenced by other sources of isotopic discrimination. Other potential sources of error in
505 interpreting stable isotope data are the values assumed for long-term average daytime
506 VPD, which are estimated from a global climate dataset (see Methods), and values
507 assumed for source air $\delta^{13}\text{C}$. Eddy flux measurements have the advantage of measuring
508 the behaviour of entire ecosystems, rather than individual leaves. However, these
509 measurements are also subject to noise, and errors may be introduced in the estimation of
510 GPP from measurements of net ecosystem CO_2 exchange (Desai et al. 2008).
511 Furthermore, eddy flux data are known to suffer from an unresolved energy balance
512 problem, in that the sum of latent and sensible heat fluxes is generally less than net
513 radiation (Wilson et al. 2002, Foken 2008). The cause of this imbalance is not yet
514 understood but may differ across sites. There are thus significant uncertainties associated
515 with each of the three datasets. It is also important to be aware of potential bias
516 introduced by different spatial coverage of the three datasets (Figure 1). While we have
517 been able to make some comparisons of different methodologies at specific sites (Figure
518 6), more such comparisons – and comparisons with isotopic data – would be valuable
519 (e.g. Monson et al. 2010).
520
521 With global change accelerating, it is more important now than ever to make use of all
522 available datasets to develop and constrain predictive models of vegetation function.

523 Cross-comparison of methodologically independent datasets, as we have done here, is a
524 crucial step forward. It highlights areas of inconsistency that should be high priorities for
525 further research. It also quantifies the uncertainty associated with different measurement
526 methods. Finally, our comparison indicates a need for understanding of potential biases
527 when using any or all of these three datasets to constrain or validate ecosystem models
528 that predict WUE.

529

530 References

531

- 532 **Barton CVM, Duursma RA, Medlyn BE, Ellsworth DS, Eamus D, Tissue DT, Adams MA,**
533 **Conroy J, Crous KY, Liberloo M, et al. 2012.** Effects of elevated atmospheric CO₂ on
534 instantaneous transpiration efficiency at leaf and canopy scales in *Eucalyptus saligna*.
535 *Global Change Biology* **18**(2): 585-595.
- 536 **Bassow S, Bazzaz F. 1999.** Canopy Photosynthesis Study at Harvard Forest 1991-1992. Harvard
537 Forest Data Archive: HF059.
- 538 **Beer C, Ciais P, Reichstein M, Baldocchi D, Law BE, Papale D, Soussana JF, Ammann C,**
539 **Buchmann N, Frank D, et al. 2009.** Temporal and among-site variability of inherent
540 water use efficiency at the ecosystem level. *Global Biogeochemical Cycles* **23**.
- 541 **Berbigier P, Bonnefond JM, Mellmann P. 2001.** CO₂ and water vapour fluxes for 2 years
542 above Euroflux forest site. *Agricultural & Forest Meteorology* **108**: 183-197.
- 543 **Bonal D, Bosc A, Ponton S, Goret JY, Burban B, Gross P, Bonnefond JM, Elbers J,**
544 **Longdoz B, Epron D, et al. 2008.** Impact of severe dry season on net ecosystem
545 exchange in the Neotropical rainforest of French Guiana. *Global Change Biology*
546 **14**(8): 1917-1933.
- 547 **Bonan GB, Oleson KW, Fisher RA, Lasslop G, Reichstein M. 2012.** Reconciling leaf
548 physiological traits and canopy flux data: Use of the TRY and FLUXNET databases in
549 the Community Land Model version 4. *Journal of Geophysical Research-Biogeosciences*
550 **117**.
- 551 **Bonan GB, Williams M, Fisher RA, Oleson KW. 2014.** Modeling stomatal conductance in the
552 earth system: linking leaf water-use efficiency and water transport along the soil-plant-
553 atmosphere continuum. *Geoscientific Model Development* **7**(5): 2193-2222.
- 554 **Bosc A. 1999.** *Etude expérimentale du fonctionnement hydrique et carboné des organes*
555 *aériens du Pin maritime (Pinus pinaster Ait.)*. PhD, Université Victor Segalen
556 Bordeaux 2.
- 557 **Buchmann N, Brooks JR, Ehleringer JR. 2002.** Predicting daytime carbon isotope ratios of
558 atmospheric CO₂ within forest canopies. *Functional Ecology* **16**(1): 49-57.
- 559 **Campany CE, Tjoelker MG, von Caemmerer S, Duursma RA. 2016.** Coupled response of
560 stomatal and mesophyll conductance to light enhances photosynthesis of shade leaves
561 under sunflecks. *Plant Cell and Environment* **39**(12): 2762-2773.
- 562 **Cernusak LA, Tcherkez G, Keitel C, Cornwell WK, Santiago LS, Knohl A, Barbour MM,**
563 **Williams DG, Reich PB, Ellsworth DS, et al. 2009.** Viewpoint: Why are non-

564 photosynthetic tissues generally C-13 enriched compared with leaves in C-3 plants?
565 Review and synthesis of current hypotheses. *Functional Plant Biology* **36**(3): 199-213.

566 **Cernusak LA, Ubierna N, Winter K, Holtum JAM, Marshall JD, Farquhar GD. 2013.**
567 Environmental and physiological determinants of carbon isotope discrimination in
568 terrestrial plants. *New Phytologist* **200**(4): 950-965.

569 **Clement RJ, Jarvis PG, Moncrieff JB. 2012.** Carbon dioxide exchange of a Sitka
570 spruce plantation in Scotland over five years. *Agricultural and Forest*
571 *Meteorology* **153**: 106-123.

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

575 **Dekker SC, Groenendijk M, Booth BBB, Huntingford C, Cox PM. 2016.** Spatial and
576 temporal variations in plant water-use efficiency inferred from tree-ring, eddy covariance
577 and atmospheric observations. *Earth System Dynamics* **7**(2): 525-533.

578 **Desai AR, Richardson AD, Moffat AM, Kattge J, Hollinger DY, Barr A, Falge E, Noormets**
579 **A, Papale D, Reichstein M, et al. 2008.** Cross-site evaluation of eddy covariance GPP
580 and RE decomposition techniques. *Agricultural and Forest Meteorology* **148**(6-7): 821-
581 838.

582 **Diefendorf AF, Mueller KE, Wing SL, Koch PL, Freeman KH. 2010.** Global patterns in leaf
583 C-13 discrimination and implications for studies of past and future climate. *Proceedings*
584 *of the National Academy of Sciences of the United States of America* **107**(13): 5738-5743.

585 **Evans JR, Sharkey TD, Berry JA, Farquhar GD. 1986. CARBON ISOTOPE**
586 **DISCRIMINATION MEASURED CONCURRENTLY WITH GAS-EXCHANGE TO**
587 **INVESTIGATE CO2 DIFFUSION IN LEAVES OF HIGHER-PLANTS. *Australian***
588 ***Journal of Plant Physiology* **13**(2): 281-292.**

589 **Farquhar GD, Ball MC, Von Caemmerer S, Roksandic Z. 1982.** EFFECT OF SALINITY
590 AND HUMIDITY ON DELTA-C-13 VALUE OF HALOPHYTES - EVIDENCE FOR
591 DIFFUSIONAL ISOTOPE FRACTIONATION DETERMINED BY THE RATIO OF
592 INTER-CELLULAR ATMOSPHERIC PARTIAL-PRESSURE OF CO2 UNDER
593 DIFFERENT ENVIRONMENTAL-CONDITIONS. *Oecologia* **52**(1): 121-124.

594 **Farquhar GD, Ehleringer JR, Hubick KT. 1989.** CARBON ISOTOPE DISCRIMINATION
595 AND PHOTOSYNTHESIS. *Annual Review of Plant Physiology and Plant Molecular*
596 *Biology* **40**: 503-537.

597 **Foken T. 2008.** The energy balance closure problem: An overview. *Ecological Applications*
598 **18**(6): 1351-1367.

599 **Frank DC, Poulter B, Saurer M, Esper J, Huntingford C, Helle G, Treydte K,**
600 **Zimmermann NE, Schleser GH, Ahlstrom A, et al. 2015.** Water-use efficiency and
601 transpiration across European forests during the Anthropocene. *Nature Climate Change*
602 **5**(6): 579-+.

603 **Ghannoum O, Evans JR, von Caemmerer S 2011.** Chapter 8 Nitrogen and Water Use
604 Efficiency of C4 Plants. In: Raghavendra AS, Sage RF eds. *C4 Photosynthesis and*
605 *Related CO2 Concentrating Mechanisms*. Dordrecht: Springer Netherlands, 129-146.

606 **Groenendijk M, Dolman AJ, van der Molen MK, Leuning R, Arneth A, Delpierre N, Gash**
607 **JHC, Lindroth A, Richardson AD, Verbeeck H, et al. 2011.** Assessing parameter
608 variability in a photosynthesis model within and between plant functional types using
609 global Fluxnet eddy covariance data. *Agricultural and Forest Meteorology* **151**(1): 22-38.

610 **Haverd V, Raupach MR, Briggs PR, Canadell JG, Isaac P, Pickett-Heaps C, Roxburgh SH,**
611 **van Gorsel E, Rossel RAV, Wang Z. 2013.** Multiple observation types reduce
612 uncertainty in Australia's terrestrial carbon and water cycles. *Biogeosciences* **10**(3): 2011-
613 2040.

614 **Henderson SA, Von Caemmerer S, Farquhar GD. 1992.** SHORT-TERM MEASUREMENTS
615 OF CARBON ISOTOPE DISCRIMINATION IN SEVERAL C4 SPECIES. *Australian*
616 *Journal of Plant Physiology* **19**(3): 263-285.

617 **Henderson S, von Caemmerer S, Farquhar GD, Wade LJ, Hammer G. 1998.** Correlation
618 between carbon isotope discrimination and transpiration efficiency in lines of the C-4
619 species *Sorghum bicolor* in the glasshouse and the field. *Australian Journal of Plant*
620 *Physiology* **25**(1): 111-123.

621 **Jarvis PG, McNaughton KG. 1986.** STOMATAL CONTROL OF TRANSPIRATION -
622 SCALING UP FROM LEAF TO REGION. *Advances in Ecological Research* **15**: 1-49.

623 **Jung M, Henkel K, Herold M, Churkina G. 2006.** Exploiting synergies of global land cover
624 products for carbon cycle modeling. *Remote Sensing of Environment* **101**(4): 534-553.

625 **Kala J, De Kauwe MG, Pitman AJ, Lorenz R, Medlyn BE, Wang YP, Lin YS, Abramowitz**
626 **G. 2015.** Implementation of an optimal stomatal conductance scheme in the Australian
627 Community Climate Earth Systems Simulator (ACCESS1.3b). *Geoscientific Model*
628 *Development* **8**(12): 3877-3889.

629 **Kala J, De Kauwe MG, Pitman AJ, Medlyn BE, Wang Y-P, Lorenz R, Perkins-Kirkpatrick**
630 **SE. 2016.** Impact of the representation of stomatal conductance on model projections of
631 heatwave intensity. *Scientific Reports* **6**.

632 **Keenan TF, Hollinger DY, Bohrer G, Dragoni D, Munger JW, Schmid HP, Richardson**
633 **AD. 2013.** Increase in forest water-use efficiency as atmospheric carbon dioxide
634 concentrations rise. *Nature* **499**(7458): 324-+.

635 **Kolari P, Lappalainen HK, Hanninen H, Hari P. 2007.** Relationship between
636 temperature and the seasonal course of photosynthesis in Scots pine at northern
637 timberline and in southern boreal zone. *Tellus Series B-Chemical and Physical*
638 *Meteorology* **59**(3): 542-552.

639 **Launiainen S, Katul GG, Kolari P, Vesala T, Hari P. 2011.** Empirical and optimal stomatal
640 controls on leaf and ecosystem level CO₂ and H₂O exchange rates. *Agricultural and*
641 *Forest Meteorology* **151**(12): 1672-1689.

642 **Law BE, Falge E, Gu L, Baldocchi DD, Bakwin P, Berbigier P, Davis K, Dolman AJ, Falk**
643 **M, Fuentes JD, et al. 2002.** Environmental controls over carbon dioxide and water vapor
644 exchange of terrestrial vegetation. *Agricultural and Forest Meteorology* **113**(1-4): 97-
645 120.

646 **Lin YS, Medlyn BE, Duursma RA, Prentice IC, Wang H, Baig S, Eamus D, de Dios VR,**
647 **Mitchell P, Ellsworth DS, et al. 2015.** Optimal stomatal behaviour around the world.
648 *Nature Climate Change* **5**(5): 459-464.

649 **Linderson ML, Mikkelsen TN, Ibrom A, Lindroth A, Ro-Poulsen H, Pilegaard K. 2012.**
650 Up-scaling of water use efficiency from leaf to canopy as based on leaf gas exchange
651 relationships and the modeled in-canopy light distribution. *Agricultural and Forest*
652 *Meteorology* **152**: 201-211.

653 **Lloyd J, Farquhar GD. 1994.** C-13 Discrimination During Co₂ Assimilation by the Terrestrial
654 Biosphere. *Oecologia* **99**(3-4): 201-215.

655 **StPaul NKM, Limousin JM, Rodriguez-Calcerrada J, Ruffault J, Rambal S, Letts MG,**

656 **Misson L. 2012.** Photosynthetic sensitivity to drought varies among populations of
657 *Quercus ilex* along a rainfall gradient. *Functional Plant Biology* **39**(1): 25-37.

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

660 **Medlyn BE, Duursma RA, Eamus D, Ellsworth DS, Prentice IC, Barton CVM, Crous KY,**
661 **de Angelis P, Freeman M, Wingate L. 2011.** Reconciling the optimal and empirical
662 approaches to modelling stomatal conductance. *Global Change Biology* **17**(6): 2134-
663 2144.

664 **Monson RK, Prater MR, Hu J, Burns SP, Sparks JP, Sparks KL, Scott-Denton LE. 2010.**
665 Tree species effects on ecosystem water-use efficiency in a high-elevation, subalpine
666 forest. *Oecologia* **162**(2): 491-504.

667 **Monteith JL. 1986.** HOW DO CROPS MANIPULATE WATER-SUPPLY AND DEMAND.
668 *Philosophical Transactions of the Royal Society a-Mathematical Physical and*
669 *Engineering Sciences* **316**(1537): 245-259.

670 **Morison JIL, Gifford RM. 1983.** STOMATAL SENSITIVITY TO CARBON-DIOXIDE AND
671 HUMIDITY - A COMPARISON OF 2 C-3 AND 2 C-4 GRASS SPECIES. *Plant*
672 *Physiology* **71**(4): 789-796.

673 **New M, Lister D, Hulme M, Makin I. 2002.** A high-resolution data set of surface climate over
674 global land areas. *Climate Research* **21**(1): 1-25.

675 **Newville M, Stensitzki T, Allen DB, Ingargiola A. 2014.** LMFIT: Non-Linear Least-Square
676 Minimization and Curve-Fitting for Python [Data set]. Zenodo.
677 <http://doi.org/10.5281/zenodo.11813>

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

681 **Op de Beek M, Low M, Deckmyn G, Ceulemans R. 2010.** A comparison of photosynthesis-
682 dependent stomatal models using twig cuvette field data for adult beech (*Fagus sylvatica*
683 *L.*). *Agricultural and Forest Meteorology* **150**(4): 531-540.

684 **Orchard KA, Cernusak LA, Hutley LB. 2010.** Photosynthesis and water-use efficiency
685 of seedlings from northern Australian monsoon forest, savanna and swamp
686 habitats grown in a common garden. *Functional Plant Biology* **37**(11): 1050-
687 1060.

688 **Rambal S, Ourcival JM, Joffre R, Mouillot F, Nouvellon Y, Reichstein M,**
689 **Rocheteau A. 2003.** Drought controls over conductance and assimilation of a
690 Mediterranean evergreen ecosystem: scaling from leaf to canopy. *Global Change*
691 *Biology* **9**(12): 1813-1824.

692 **Saurer M, Spahni R, Frank DC, Joos F, Leuenberger M, Loader NJ, McCarroll D, Gagen**
693 **M, Poulter B, Siegwolf RTW, et al. 2014.** Spatial variability and temporal trends in
694 water-use efficiency of European forests. *Global Change Biology* **20**(12): 3700-3712.

695 **Seibt U, Rajabi A, Griffiths H, Berry JA. 2008.** Carbon isotopes and water use efficiency:
696 sense and sensitivity. *Oecologia* **155**(3): 441-454.

697 **Sinclair TR, Tanner CB, Bennett JM. 1984.** WATER-USE EFFICIENCY IN CROP
698 PRODUCTION. *Bioscience* **34**(1): 36-40.

699 **Tarvainen L, Wallin G, Rantfors M, Uddling J. 2013.** Weak vertical canopy gradients of
700 photosynthetic capacities and stomatal responses in a fertile Norway spruce stand.
701 *Oecologia* **173**(4): 1179-1189.

702 **Thom AS. 1972.** MOMENTUM, MASS AND HEAT-EXCHANGE OF VEGETATION.
703 *Quarterly Journal of the Royal Meteorological Society* **98**(415): 124-&
704 **Urbanski S, Barford C, Wofsy S, Kucharik C, Pyle E, Budney J, McKain K,**
705 **Fitzjarrald D, Czikowsky M, Munger JW. 2007.** Factors controlling CO₂
706 exchange on timescales from hourly to decadal at Harvard Forest. *Journal of*
707 *Geophysical Research-Biogeosciences* **112**(G2).
708 **Vesala T, Suni T, Rannik U, Keronen P, Markkanen T, Sevanto S, Gronholm T,**
709 **Smolander S, Kulmala M, Ilvesniemi H, et al. 2005.** Effect of thinning on surface
710 fluxes in a boreal forest. *Global Biogeochemical Cycles* **19**(2).
711 **Wehr R, Munger JW, McManus JB, Nelson DD, Zahniser MS, Davidson EA, Wofsy SC,**
712 **Saleska SR. 2016.** Seasonality of temperate forest photosynthesis and daytime
713 respiration. *Nature* **534**(7609): 680-+.
714 **Wehr R, Commane R, Munger JW, McManus JB, Nelson DD, Zahniser MS,**
715 **Saleska SR, Wofsy SC. 2017.** Dynamics of canopy stomatal conductance,
716 **transpiration, and evaporation in a temperate deciduous forest, validated by**
717 **carbonyl sulfide uptake. *Biogeosciences* 14(2): 389-401.**
718 **Wilson K, Goldstein A, Falge E, Aubinet M, Baldocchi D, Berbigier P, Bernhofer C,**
719 **Ceulemans R, Dolman H, Field C, et al. 2002.** Energy balance closure at FLUXNET
720 sites. *Agricultural and Forest Meteorology* **113**(1-4): 223-243.
721 **Wingate L, Seibt U, Moncrieff JB, Jarvis PG, Lloyd J. 2007.** Variations in (13)C
722 discrimination during CO₂ exchange by *Picea sitchensis* branches in the field.
723 *Plant Cell and Environment* **30**(5): 600-616.
724 **Zhou S, Yu B, Huang YF, Wang GQ. 2015.** Daily underlying water use efficiency for
725 **AmeriFlux sites. *Journal of Geophysical Research-Biogeosciences* 120(5): 887-902.**

726 **Zhou S, Yu BF, Zhang Y, Huang YF, Wang GQ. 2016.** Partitioning evapotranspiration
727 based on the concept of underlying water use efficiency. *Water Resources*
728 *Research* **52**(2): 1160-1175.
729

730

731 **Acknowledgements**

732 This work was funded by ARC Discovery Grant DP120104055. This work used eddy
733 covariance data acquired by the FLUXNET community and in particular by the following
734 networks: AmeriFlux (U.S. Department of Energy, Biological and Environmental
735 Research, Terrestrial Carbon Program (DE-FG02-04ER63917 and DE-FG02-
736 04ER63911)), AfriFlux, AsiaFlux, CarboAfrica, CarboEuropeIP, CarboItaly, CarboMont,
737 ChinaFlux, Fluxnet-Canada (supported by CFCAS, NSERC, BIOCAP, Environment
738 Canada, and NRCan), GreenGrass, KoFlux, LBA, NECC, OzFlux, TCOS-Siberia,
739 USCCC. We acknowledge the financial support to the eddy covariance data
740 harmonization provided by CarboEuropeIP, FAO-GTOS-TCO, iLEAPS, Max Planck
741 Institute for Biogeochemistry, National Science Foundation, University of Tuscia,
742 Université Laval and Environment Canada and US Department of Energy and the
743 database development and technical support from Berkeley Water Center, Lawrence
744 Berkeley National Laboratory, Microsoft Research eScience, Oak Ridge National
745 Laboratory, University of California - Berkeley, University of Virginia. We thank Wang
746 Han for extracting the VPD data corresponding to isotope data. We thank Will Cornwell,
747 Trevor Keenan, Colin Prentice and Ian Wright for valuable discussions on this topic.

748

749

750

751 **Author Contributions**

752 BEM and RAD conceived and designed the study. BEM led writing of paper. MDK and
753 YSL assembled and processed datasets, with assistance from JK, RAD and CAW. AA,
754 RC, PI, J-ML, M-LL, PM, N M-S, and LW assisted with interpretation of datasets. All
755 authors contributed to writing of paper.

756

757 **Author Information**

758

759 Data deposition statement: All data and code are available online as follows.

760 Leaf gas exchange dataset: <https://bitbucket.org/gsglobal/leafgasexchange>

761 Stable isotope dataset: Dryad Digital Repository. <http://dx.doi.org/10.5061/dryad.3jh61>
762 (Currently under embargo: this address will become live once Cornwell et al. in review
763 paper is accepted)

764 Eddy covariance dataset: <http://fluxnet.fluxdata.org/data/la-thuile-dataset/>

765 Analysis code: https://github.com/mdekauwe/gl_leaf_canopy_ecosystem

766

767 The authors declare no competing financial interests.

768

769 Correspondence and requests for materials should be addressed to

770 b.medlyn@westernsydney.edu.au

771

772

773

774

775 **Table 1: Significant differences among PFTs by Method**

776

777 Linear mixed models with site as a random factor were applied to gas exchange, isotope
 778 and flux data sets separately, and Tukey’s HSD used to determine significant differences
 779 across PFTs. PFTs with different letters for a given measurement type are significantly
 780 different for that measurement type: for example, in the “gas exchange” column, ENF
 781 (letter ‘a’) is significantly different from DBF (letters ‘bc’) but not EBF (letters ‘ac’).
 782 Isotope values were log-transformed before analysis. Values of n in table indicate number
 783 of sites used for each PFT.

784

PFT	Gas exchange	n	Isotope	n	Flux	n
ENF (evergreen needleleaf forest)	a	13	a	85	cd	38
EBF (evergreen broadleaf forest)	ac	9	bd	139	bd	7
DBF (deciduous broadleaf forest)	bc	12	bc	108	bc	17
TRF (tropical rainforest)	ab	4	e	95	abd	1
SAV (savanna)	bc	7	de	31	bd	6
SHB (shrub)	ab	6	cd	215	d	4
C3G (C3 grass)	b	2	b	208	d	25
C4G (C4 grass)	a	5	-	-	-	-
C3C (C3 crops)	bc	4	-	-	b	15
C4C (C4 crops)	-	-	-	-	a	7

785

786

787 **Table 2: Datasets used for leaf-canopy comparison at individual sites**

788 Details of FLUXNET sites and leaf gas exchange datasets used for leaf-canopy comparison shown in Figure 6.

789

FLUXNET Site ID	Lat.	Long.	FLUXNET time period	Gas exchange sampling	FLUXNET reference	Gas exchange reference
AU-Tum	-35.66	148.15	12,1,3 / 2002	Diurnal spot measurements, mid-canopy, 3 campaigns (Nov-01, Feb-02, May-02)	Leuning et al. (2005)	Medlyn et al. (2007)
DK-Sor	55.49	12.10	5,6,7 / 1999	Diurnal spot measurements, upper canopy, 11 dates during Jun-Aug 99	Pilegaard et al. (2003)	Linderson et al. (2012)
FI-Hyy	61.85	24.29	5,6,7 / 2006	Automated shoot cuvette, upper canopy, continuous measurements, Jul-06	Vesala et al. (2005)	Kolari et al. (2007)
FR-LBr	44.72	-0.77	6,7,8 / 1997	Automated branch cuvette, upper canopy, continuous measurements, Sep-97	Berbigier et al. (2001)	Bosc (1999)
Fr-Pue	43.74	3.60	5,6,10 / 2006	First point of A-Ci curves, upper canopy, 11 dates during Apr-Dec 09	Rambal et al. (2003)	Martin-StPaul et al. (2012)
GF-Guy	5.28	-52.93	6,7,8 / 2006	Light-saturated photosynthesis, upper canopy, Oct-10	Bonal et al. (2008)	J. Zaragoza-Castells, O. Atkin, P. Meir, pers. comm.
UK-Gri	56.61	-0.86	5,6,7 / 2001	Automated branch cuvette, upper and mid-canopy, Jul-01	Clement et al. (2003)	Wingate et al. (2007)
US-Ha1	42.54	-72.17	6,7,8 / 1992	Diurnal spot measurements, upper canopy, monthly Jun-Sep 91/92	Urbanski et al. (2007)	Bassow & Bazzaz (1999)

790

791

792 **Figure Captions**

793

794 **Figure 1:** Global distribution of datasets used in the study.

795

796 **Figure 2:** Box and whisker plot (line, median; box, inter-quartile range) showing the
797 estimated g_1 values from leaf gas exchange, leaf isotope and FLUXNET data, grouped by
798 plant functional type. Whiskers extend to 1.5 times the inter-quartile range, with dots
799 outside of the whiskers showing outliers. Plant functional types are defined as: ENF -
800 evergreen needleleaf forest, EBF - evergreen broadleaf forest, DBF - deciduous broadleaf
801 forest, TRF - tropical rainforest, SAV - savanna, SHB - shrub, C3G – C3 grass, C4G –
802 C4 grass, C3C – C3 crops, C4C – C4 crops. Values of n indicate no of species for leaf
803 gas exchange and leaf isotope datasets, and no of site-years for FLUXNET. Different
804 letters below boxes denote significant differences among methods for each PFT (Tukey's
805 Honest Significant Difference test, $p < 0.05$). Data shown have been clipped to a
806 maximum g_1 of 14, which excludes 0.0%, 3.18% and 0.22% of leaf gas exchange, leaf
807 isotope and FLUXNET datasets, respectively.

808

809 **Figure 3:** Values of g_{1-flux} for grasslands as a function of the estimated fraction of C4
810 vegetation.

811

812 **Figure 4:** Values of g_{1-flux} for forest and non-forest vegetation as a function of peak LAI.

813

814 **Figure 5:** Estimated g_1 values from leaf gas exchange, leaf isotope and FLUXNET data,
815 shown as a function of latitude. Where several values were obtained at the same site
816 (different species for leaf gas exchange and isotope, different years for Fluxnet), values
817 have been averaged and standard error bars show variability. Plant functional types are
818 defined as: ENF - evergreen needleleaf forest, EBF - evergreen broadleaf forest, DBF -
819 deciduous broadleaf forest, TRF - tropical rainforest, SAV - savanna, SHB - shrub, C3G
820 – C3 grass, C4G – C4 grass, C3C – C3 crops, C4C – C4 crops. Data shown have been
821 clipped to a maximum g_1 of 14.

822

823 **Figure 6:** Comparison for individual sites between measured leaf-scale stomatal
824 conductance and canopy conductance estimated from FLUXNET as a function of a
825 stomatal index (for gas exchange: $A / (C_a \sqrt{D})$ and for FLUXNET: $GPP / (C_a \sqrt{D})$).
826 Background points show data, darker points show fitted values. Details of gas exchange
827 and FLUXNET measurements are given in Table 2. Measurements were taken from the
828 same year whenever overlapping data were available. The g_1 values shown are the values
829 fitted to the corresponding data.

830

831 **Figure 7:** Box and whisker plot (line, median; box, inter-quartile range) showing the
832 estimated g_1 values from leaf gas exchange, and FLUXNET data calculated using eqn (4)
833 to estimate canopy stomatal conductance (FLUXNET) or the Penman-Monteith equation
834 (eqn 5, FLUXNET-PM). The Fluxnet data are a subset of the data shown in Figure 1 and
835 include only those sites for which eqn 5 could be applied. Whiskers extend to 1.5 times
836 the inter-quartile range, with dots outside of the extent of the whiskers showing outlying
837 values. Plant functional types are defined as: ENF - evergreen needleleaf forest, EBF -
838 evergreen broadleaf forest, DBF - deciduous broadleag forest, TRF - tropical rainforest,
839 SAV - savanna, SHB - shrub, C3G - C3 grass, C4G - C4 grass, C3C - C3 crops, C4C -
840 C4 crops.

841

842