

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

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## Summary

- The terrestrial carbon and water cycles are intimately linked: the carbon cycle is driven by photosynthesis, while the water balance is dominated by transpiration, and both fluxes are controlled by plant stomatal conductance. The ratio between these fluxes, the plant water-use efficiency (WUE), is a useful indicator of vegetation function.
- WUE can be estimated using several techniques, including leaf gas exchange, stable isotope discrimination, and eddy covariance. Here we compare global compilations of data for each of these three techniques.
- We show that patterns of variation in WUE across plant functional types (PFTs) are not consistent among the three datasets. Key discrepancies include the following: leaf-scale data indicate differences between needleleaf and broadleaf forests, but ecosystem-scale data do not; leaf-scale data indicate differences between C<sub>3</sub> and C<sub>4</sub> species, whereas at ecosystem scale there is a difference between C<sub>3</sub> and C<sub>4</sub> crops but not grasslands; and isotope-based estimates of WUE are higher than estimates based on gas exchange for most PFTs.
- Our study quantifies the uncertainty associated with different methods of measuring WUE, indicates potential for bias when using WUE measures to parameterize or validate models, and indicates key research directions needed to reconcile alternative measures of WUE.

## Introduction

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

Water-use efficiency can be estimated using several methods that operate at different temporal and spatial scales. Community

research efforts have led to the compilation of global datasets based on each of these methods. These datasets are increasingly being utilized to constrain and evaluate global vegetation models (e.g. Groenendijk *et al.*, 2011; Saurer *et al.*, 2014; Kala *et al.*, 2015; Dekker *et al.*, 2016). However, to date there has been little comparison across methods. It is often assumed that values obtained at one scale should be relatable to values obtained at other scales, but this assumption has not been explicitly tested across ecosystems. Our goal in this paper is to compare three independent global datasets of WUE, obtained using leaf gas exchange, stable isotope, and eddy covariance techniques, and to investigate whether global patterns obtained using these different techniques are consistent with our current understanding of scaling. Specifically, we focus on patterns of variation across plant

functional types (PFTs), which are used to represent vegetation in global vegetation models, and ask whether the three datasets indicate consistent differences among PFTs.

Water-use efficiency is known to vary with atmospheric vapour pressure deficit (VPD) (Monteith, 1986). To compare across datasets, a metric of WUE is required that accounts for this variation. One commonly used metric is the intrinsic WUE (iWUE), defined as photosynthetic C uptake divided by stomatal conductance to water vapour ( $A/g_s$ ). Another related metric is the ratio of intercellular to atmospheric  $\text{CO}_2$  ( $C_i : C_a$ ). However, both iWUE and the  $C_i : C_a$  ratio also vary with VPD, meaning that values obtained under different VPD conditions cannot be directly compared. In this work, we account for variation in VPD conditions by using the parameter  $g_1$  of a recent model of stomatal conductance ( $g_s \text{ mol m}^{-2} \text{ s}^{-1}$ ), derived from the theory of optimal stomatal behaviour (Medlyn *et al.*, 2011):

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

where  $A$  is the net assimilation rate ( $\mu\text{mol m}^{-2} \text{ s}^{-1}$ ), and  $C_s$  ( $\mu\text{mol mol}^{-1}$ ) and  $D$  (kPa) are the  $\text{CO}_2$  concentration and VPD at the leaf surface, respectively. The model parameter  $g_1$  ( $\text{kPa}^{0.5}$ ) represents normalized plant WUE. The model parameter  $g_1$  is inversely related to iWUE but accounts for VPD by assuming a  $\sqrt{D}$  dependence of the  $C_i : C_a$  ratio, as found for leaf gas exchange (Medlyn *et al.*, 2011) and eddy covariance data (Zhou *et al.*, 2015). This parameter also corrects for increases in WUE driven by changes in  $C_a$ . If the ratio  $C_i : C_a$  is constant with increasing  $C_a$ , then  $g_1$  is also constant (Medlyn *et al.*, 2011). Assuming that these relationships accurately account for environmental effects on WUE, the parameter  $g_1$  is then a measure of WUE that can be directly compared across datasets.

We apply this model to three major global data compilations. Lin *et al.* (2015) compiled a global database of leaf gas exchange measurements, including photosynthetic rate and stomatal conductance, and used these data to estimate instantaneous values of  $g_1$ . Lin *et al.* (2015) found systematic differences in  $g_1$  among PFTs, with high values of  $g_1$  (and thus low iWUE) in crops,  $C_3$  grasses and deciduous angiosperm trees, and low values in  $C_4$  grasses and gymnosperms. Leaf-level gas exchange data such as these are commonly used to parameterize stomatal behaviour in vegetation models (e.g. Bonan *et al.*, 2014). The differences among PFTs observed by Lin *et al.* (2015) have important consequences for modelled vegetation function at large scales, including changes in predicted surface cooling and consequent heatwave development (Kala *et al.*, 2015, 2016).

Stable isotope methods can be applied to plant tissue to estimate iWUE and  $g_1$  values over monthly to annual timescales (Farquhar *et al.*, 1989; Cernusak *et al.*, 2013). Long-term stable isotope records from tree rings are widely used to constrain model predictions of WUE at large spatial and temporal scales (e.g. Saurer *et al.*, 2014; Frank *et al.*, 2015; Dekker *et al.*, 2016). A compilation of leaf  $^{13}\text{C}$  discrimination measurements indicated differences in stomatal behaviour among PFTs (Diefendorf *et al.*,

2010). Here, we estimated  $g_1$  values from a global database of nearly 4000 measurements of bulk leaf  $^{13}\text{C}$  discrimination ( $\Delta^{13}\text{C}$ ), taken from 594 sites spread across all seven continents (Cornwell *et al.*, 2017). We predicted that values of  $g_1$  estimated from this dataset would show similar rankings across PFTs as the leaf gas exchange data set, but that values would be lower, as a result of mesophyll resistance to  $\text{CO}_2$  diffusion (Seibt *et al.*, 2008).

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

## Materials and Methods

### Datasets

We synthesized three independent datasets to estimate values of  $g_1$ . All datasets and our analysis code are available online; web addresses are given below under 'data deposition statement'. Leaf gas exchange data were taken from Lin *et al.* (2015), who collated measurements under ambient field conditions from 286 species, covering 56 sites across the globe. The majority of these data are measurements on upper-canopy leaves during the growing season. Isotope data came from a global database of leaf carbon isotopes measurements from natural and seminatural habitats, across 3985 species–site combinations (Cornwell *et al.*, 2017). Flux measurements were taken from the global collection of eddy flux measurements that comprise the FLUXNET 'La Thuile' Free and Fair dataset (<http://www.fluxdata.org>). This dataset contains gap-filled, half-hourly measurements of carbon dioxide, water vapour and energy fluxes; following filtering (see later) we were able to use data from 120 sites. The global distribution of the three datasets is shown in Fig. 1.

### Estimating $g_1$

The value of  $g_1$  was estimated from leaf gas exchange data using nonlinear regression to fit the unified stomatal optimization model (Medlyn *et al.*, 2011; Eqn 1) to  $g_s$  measurements for each species. Here we followed the methods of Lin *et al.* (2015). All model fits were done using the 'minimize' function of the python 'lmfit' library, using the Levenberg–Marquardt method (Newville *et al.*, 2014).

Cornwell *et al.* (2017) estimated carbon isotope discrimination ( $\Delta$ ) values from bulk leaf  $\delta^{13}\text{C}$  and estimates of source air  $\delta^{13}\text{C}$  composition. From these data, we estimated the ratio of the

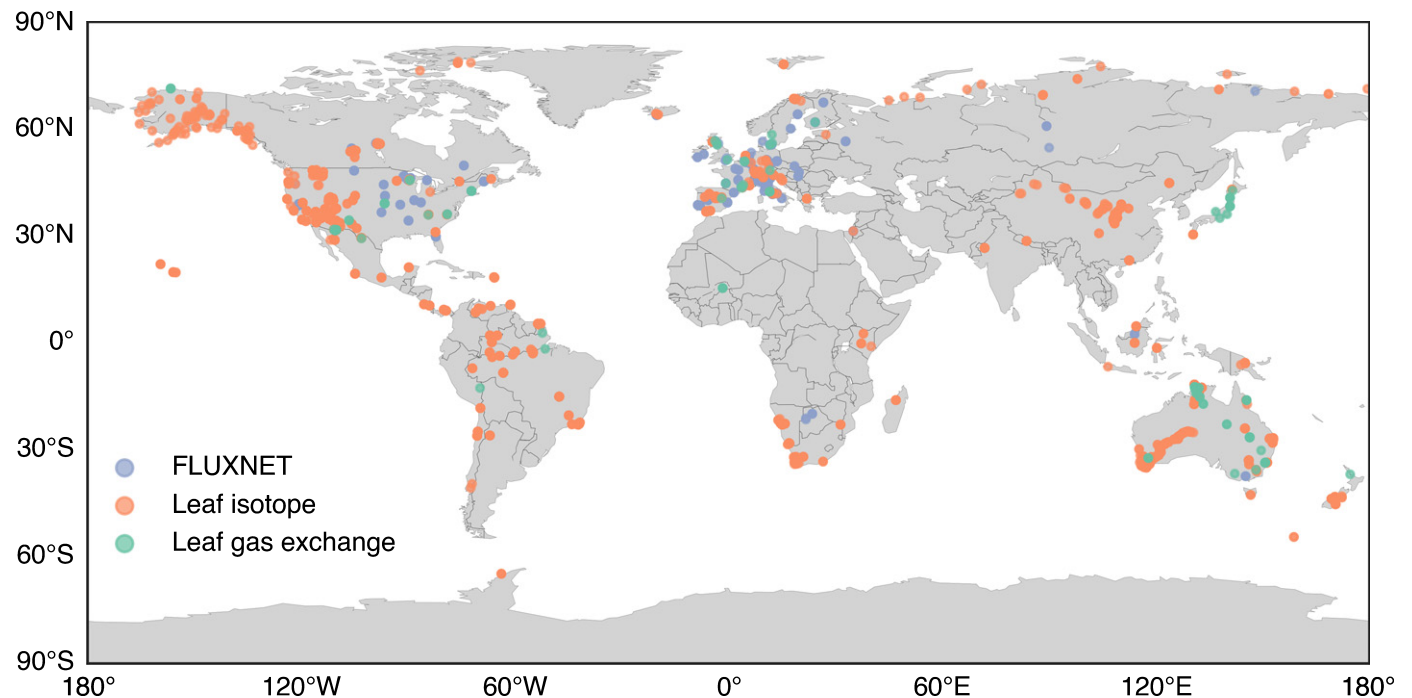


Fig. 1 Global distribution of datasets used in the study.

intercellular to ambient carbon dioxide concentration ( $C_i : C_a$ ) following Farquhar *et al.* (1989) for  $C_3$  species:

$$\frac{C_i}{C_a} = \frac{\Delta - a}{b - a} \quad \text{Eqn 2}$$

where  $a$  represents the fractionation caused by gaseous diffusion (4.4‰) and  $b$  is the effective fractionation caused by carboxylating enzymes (assumed to be 27‰) (Cernusak *et al.*, 2013). Note that we were unable to utilize values for  $C_4$  vegetation from this dataset. For  $C_4$  plants, the relationship between  $C_i : C_a$  and  $\Delta^{13}\text{C}$  depends on bundle sheath leakiness,  $\phi$  (Henderson *et al.*, 1998; Cernusak *et al.*, 2013). Adopting a value for  $\phi$  of 0.21 for  $C_4$  vegetation, as suggested by Henderson *et al.* (1998), yielded unrealistic estimates of  $C_i : C_a < 0$  for more than half (79/140) of the dataset.

Values of  $g_1$  for  $C_3$  species were estimated following Medlyn *et al.* (2011):

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

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

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

$$G_s = \frac{LE/\lambda}{D/P} \quad \text{Eqn 4}$$

where  $\lambda$  is the latent heat of water vapour ( $\text{J mol}^{-1}$ ),  $D$  (Pa) is the VPD and  $P$  is the atmospheric pressure (Pa). Pressure was estimated using the hypsometric equation based on site elevation data. Where site elevation information was missing, values were gap-filled using the 30-arc seconds (~1 km) global digital elevation model GTOPO30 data from the United States Geological Survey (USGS). Values of  $g_1$  were then estimated by fitting Eqn 1 to data, taking  $G_s$  for  $g_s$  and GPP for  $A$ .

FLUXNET data were screened as follows: (1) data flagged as 'good'; (2) data from the three most productive months, in terms of flux-derived GPP (to account for the different timing of summer in the northern and southern hemispheres); (3) daylight hours between 09:00 and 15:00 h; (4) time slices with precipitation, as well as the subsequent 48 half-hour time slices, were excluded (to minimize contributions from soil/wet canopy evaporation); (5) time slices with missing  $\text{CO}_2$  data were gap-filled with the global annual mean from averaged marine surface (<http://www.esrl.noaa.gov/gmd/ccgg/trends/>). If the entire year's data were missing, or if the annual mean departed from the global mean by  $\pm 15\%$ , data were replaced with the global mean. This screening check was used to address possible errors in locally recorded  $\text{CO}_2$  concentrations in 14 site-year combinations, which showed drops against a global trend of increasing  $\text{CO}_2$  concentrations (1995–2004:  $1.87 \text{ ppm yr}^{-1}$ ). In addition, fitted  $g_1$  values with an  $R^2 < 0.2$

were excluded, as were fitted  $g_1$  values that were  $\pm 50\%$  from the site average.

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

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

where  $G_a$  ( $\text{mol m}^{-2} \text{s}^{-1}$ ) is the canopy aerodynamic conductance,  $\lambda$  is the latent heat of water vapour ( $\text{J mol}^{-1}$ ),  $E$  ( $\text{mol m}^{-2} \text{s}^{-1}$ ) is the canopy transpiration,  $\gamma$  is the psychrometric constant ( $\text{Pa K}^{-1}$ ),  $s$  is the slope of the saturation vapour pressure curve at air temperature ( $\text{Pa K}^{-1}$ ),  $R_n$  ( $\text{W m}^{-2}$ ) is the net radiation,  $D$  ( $\text{Pa}$ ) is the VPD,  $G$  ( $\text{W m}^{-2}$ ) is the soil heat flux,  $M_a$  ( $\text{kg mol}^{-1}$ ) is molar mass of air, and  $c_p$  is the heat capacity of air ( $\text{J kg}^{-1} \text{K}^{-1}$ ). At sites where values of  $G$  were not available,  $G$  was set to zero.  $G_a$  was calculated as  $P/(R_{\text{gas}} T_k)/(u/u^* + 6.2u^{*-2/3})$ , where  $u^*$  ( $\text{m s}^{-1}$ ) is friction velocity and  $u$  ( $\text{m s}^{-1}$ ) is wind speed (Thom, 1972).  $P$  is atmospheric pressure ( $\text{Pa}$ ),  $R_{\text{gas}}$  is the gas constant ( $\text{J mol}^{-1} \text{K}^{-1}$ ),  $T_k$  is the air temperature in Kelvin, and the term  $P/(R_{\text{gas}} T_k)$  converts from units of  $\text{m s}^{-1}$  to  $\text{mol m}^{-2} \text{s}^{-1}$ . Eqn 5 was applied to all datasets where  $R_n$  and  $u^*$  were available. Inspection of Eqn 5 shows that, under most conditions, incorporating a finite  $G_a$  value will lead to a lower estimate of  $G_c$  than would be obtained with infinite  $G_a$ .

### Ancillary data

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

Estimates of the relative fraction of  $C_4$  present at each FLUXNET site were derived from the closest matching 0.5-degree pixel in the North American Carbon Program (NACP) Global  $C_3$  and  $C_4$  SYNergetic land cover MAP (SYNMAP) (Jung *et al.*, 2006).

Peak leaf area index (LAI) for FLUXNET sites was obtained from the site-level ancillary data when available in the supporting documents contributed to the La Thuile Synthesis Collection (see [www.fluxdata.org](http://www.fluxdata.org)).

### Statistics

We tested for statistical differences among methods by applying one-way ANOVA to log-transformed values of  $g_{1\text{-leaf}}$ ,  $g_{1\text{-isotope}}$  and site-averaged  $g_{1\text{-flux}}$  for each PFT. For each method, we used a mixed-model approach to test for differences among PFTs, taking site as a random factor. Similarly, a mixed-model approach was used to test for statistical differences among PFTs for a given method. Differences among methods and among PFTs were identified using Tukey's honest significant difference.

### Data deposition

All data and code are available online as follows.

- Leaf gas exchange dataset: <https://bitbucket.org/gsglobal/leafgasexchange>
- Stable isotope dataset: <https://github.com/wcornwell/leaf13C>
- Eddy covariance dataset: <http://fluxnet.fluxdata.org/data/la-thuille-dataset/>
- Analysis code: [https://github.com/mdekauwe/g1\\_leaf\\_canopy\\_ecosystem](https://github.com/mdekauwe/g1_leaf_canopy_ecosystem)

### Results

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

### Forest PFTs

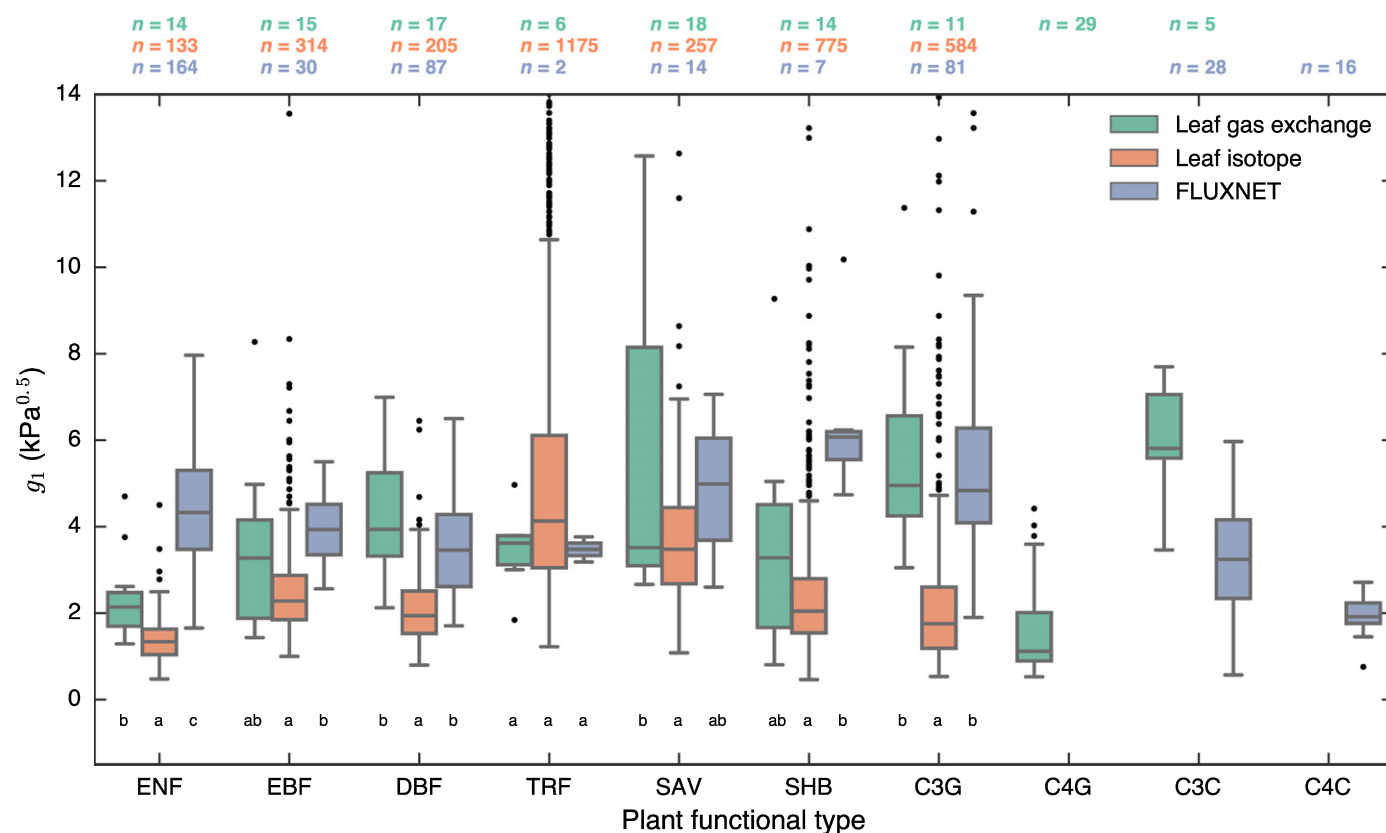
Among the four forest PFTs, median values of  $g_1$  derived from leaf gas exchange ( $g_{1\text{-leaf}}$ ) were lowest in evergreen needleleaf forest (ENF), intermediate in evergreen broadleaf forest (EBF) and highest in deciduous broadleaf forest (DBF) and tropical rainforest (TRF). Isotope-derived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

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

### Nonforest PFTs

Among the nonforest PFTs,  $g_{1\text{-leaf}}$  values were significantly higher in  $C_3$  grasses (C3G) than in  $C_4$  grasses (C4G), intermediate in





**Fig. 2** Box and whisker plot (line, median; box, interquartile range) showing the estimated  $g_1$  values from leaf gas exchange, leaf isotope and FLUXNET data, grouped by plant functional type (PFT). Whiskers extend to 1.5 times the interquartile range, with dots outside of the whiskers showing outliers. PFTs are defined as follows: ENF, evergreen needleleaf forest; EBF, evergreen broadleaf forest; DBF, deciduous broadleaf forest; TRF, tropical rainforest; SAV, savannah; SHB, shrub; C3G, C<sub>3</sub> grass; C4G, C<sub>4</sub> grass; C3C, C<sub>3</sub> crops; C4C, C<sub>4</sub> crops. Values of  $n$  indicate the number of species for leaf gas exchange and leaf isotope datasets, and number of site-years for FLUXNET. Different letters below boxes denote significant differences among methods for each PFT (Tukey's honest significant difference test,  $P < 0.05$ ). Data shown have been clipped to a maximum  $g_1$  of 14, which excludes 0.0%, 3.18% and 0.22% of leaf gas exchange, leaf isotope and FLUXNET datasets, respectively.

shrubs (SHB), and rather variable in savannah (SAV) trees. The variability of  $g_{1-\text{leaf}}$  in SAV is probably related to the high seasonality in these systems: this instantaneous measure of WUE can vary considerably between wet and dry seasons. Note that the comparison among methods for the SAV PFT is somewhat biased because eddy covariance data are from the whole ecosystem and thus include both trees and understorey, whereas leaf gas exchange for this PFT is from trees only, while isotope data are principally from trees and shrubs. As with forest PFTs, values of  $g_{1-\text{isotope}}$  for nonforest PFTs were on average lower than values of  $g_{1-\text{leaf}}$ , but the rankings of PFTs differed: C<sub>3</sub> grasses had lower  $g_{1-\text{isotope}}$  values than SAV or SHB, an unexpected result. We were unable to estimate values of  $g_{1-\text{isotope}}$  for C<sub>4</sub> species (see the Materials and Methods section) although  $\Delta^{13}\text{C}$  values clearly differed between C<sub>3</sub> and C<sub>4</sub> vegetation (Cornwell *et al.*, 2017).

Photosynthetic pathway had a significant effect on  $g_{1-\text{flux}}$  values for crop vegetation:  $g_{1-\text{flux}}$  was significantly lower in C<sub>4</sub> crops (C4C) than in C<sub>3</sub> crops (C3C). Values of  $g_{1-\text{flux}}$  were high for grasslands (C3G), similar to  $g_{1-\text{leaf}}$  values and much higher than  $g_{1-\text{isotope}}$  values. We did not find evidence that the presence of C<sub>4</sub> grasses reduced  $g_{1-\text{flux}}$  in grasslands (Fig. 3); grassland  $g_{1-\text{flux}}$  values were not correlated with estimated C<sub>4</sub> fraction.

### Comparison of forest and nonforest PFTs

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

### Exploration of inconsistent patterns among datasets

The lack of difference among  $g_{1-\text{flux}}$  values for forest PFTs was unexpected. The consistent evidence from  $g_{1-\text{leaf}}$  and  $g_{1-\text{isotope}}$  values suggests that leaf-scale  $g_1$  is low for ENF. We had anticipated that this difference would scale to canopy behaviour, yet there is

**Table 1** Significant differences among plant functional types (PFTs) by method

PFT	Gas exchange	<i>n</i>	Isotope	<i>n</i>	Flux	<i>n</i>
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 (savannah)	bc	7	de	31	bd	6
SHB (shrub)	ab	6	cd	215	d	4
C3G (C <sub>3</sub> grass)	b	2	b	208	d	25
C4G (C <sub>4</sub> grass)	a	5	–	–	–	–
C3C (C <sub>3</sub> crops)	bc	4	–	–	b	15
C4C (C <sub>4</sub> crops)	–	–	–	–	a	7

Linear mixed models with site as a random factor were applied to gas exchange, isotope and flux datasets separately, and Tukey's honest significant difference was used to determine significant differences across PFTs. PFTs with different letters for a given measurement type are significantly different for that measurement type: for example, in the 'gas exchange' column, ENF (letter 'a') is significantly different from DBF (letters 'bc') but not EBF (letters 'ac'). Isotope values were log-transformed before analysis. Values of *n* in table indicate number of sites used for each PFT.

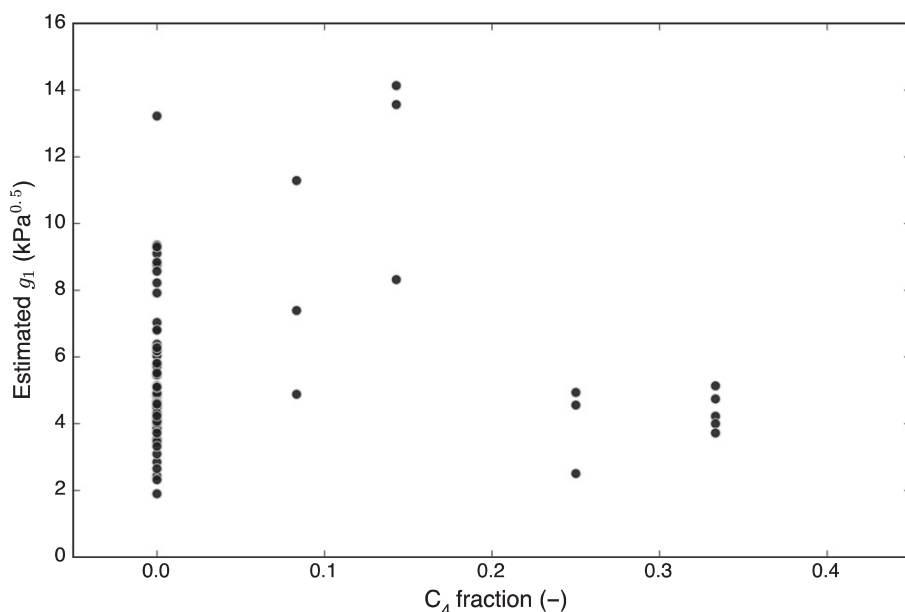
no evidence that  $g_{1-\text{flux}}$  values were lower for this PFT. It is possible that sampling biases led to different results for the three methodologies. To investigate this possibility, we first compared the latitudinal distributions of the three datasets, using latitude as an indicator of climatic conditions (Fig. 5). Clear differences in sampling coverage with latitude can be seen. However, Fig. 5 demonstrates that, irrespective of latitude, values 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 in ENF and DBF.

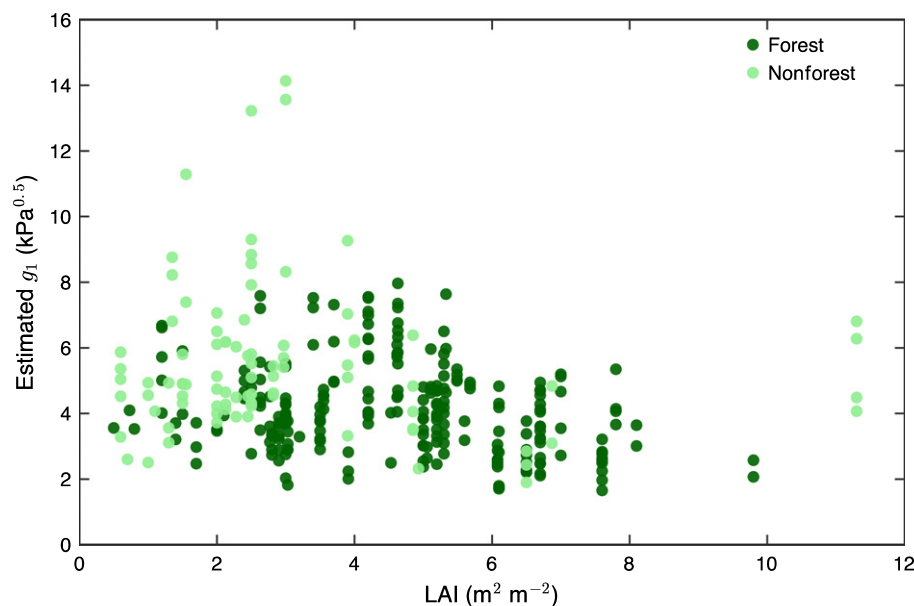
To further rule out sampling bias, we also compared half-hourly leaf gas exchange data and eddy flux data for eight sites where both kinds of data were available (Fig. 6; Table 2). This direct comparison shows that  $g_{1-\text{leaf}}$  and  $g_{1-\text{flux}}$  values were in a similar range for DBF and TRF forest types but that  $g_{1-\text{leaf}}$  was lower than  $g_{1-\text{flux}}$  for EBF and ENF forest types, further confirming that the discrepancy between  $g_{1-\text{leaf}}$  and  $g_{1-\text{flux}}$  is not simply a result of sampling bias.

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

## Discussion

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

**Fig. 3** Values of  $g_{1-\text{flux}}$  for grasslands as a function of the estimated fraction of C<sub>4</sub> vegetation.



**Fig. 4** Values of  $g_{1\text{-flux}}$  for forest and nonforest vegetation as a function of peak leaf area index (LAI).

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

#### Cross-PFT patterns compared among datasets

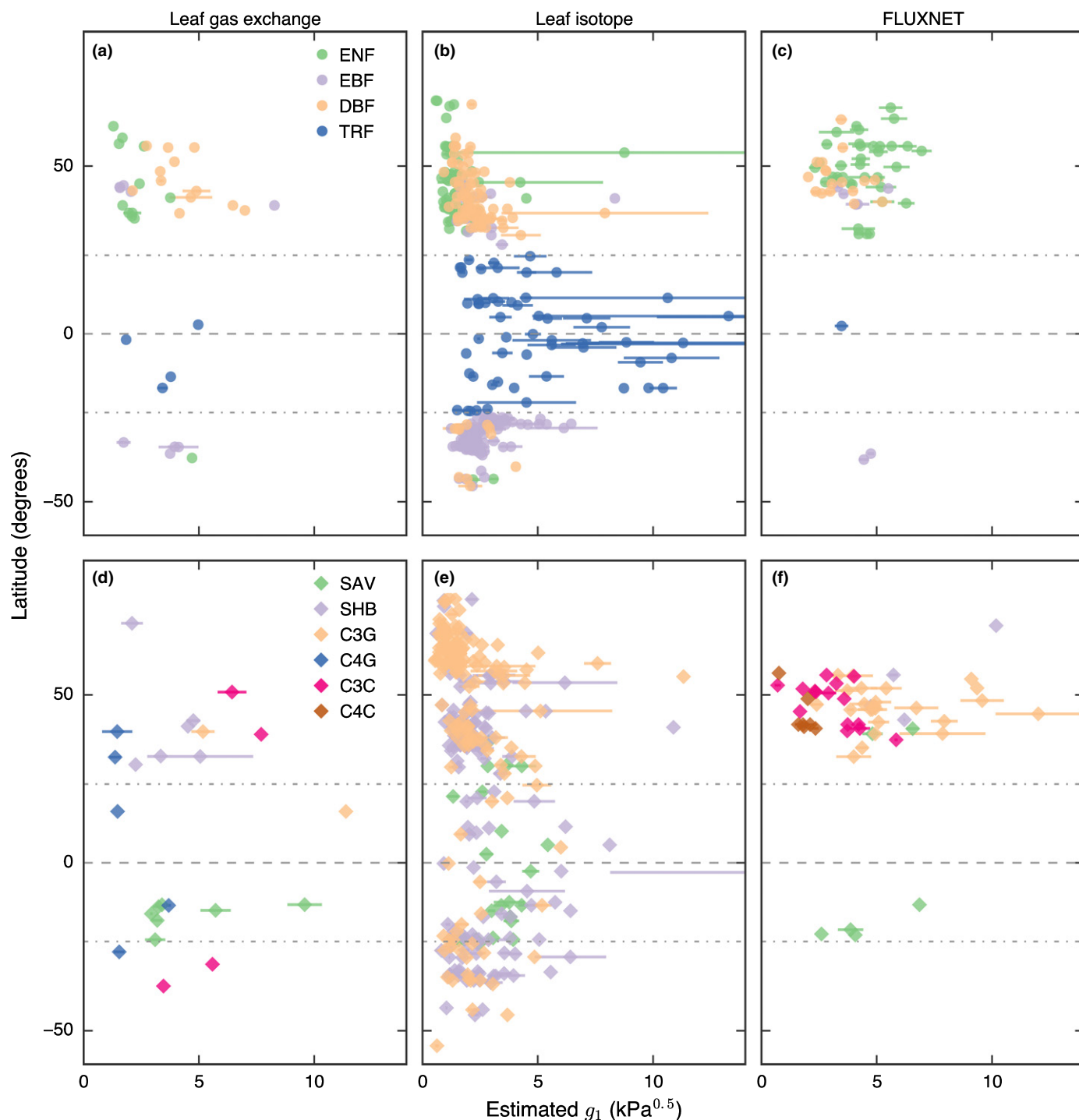
For forest vegetation, there was an important discrepancy in cross-PFT patterns between leaf and ecosystem-scale estimates of  $g_1$ . At leaf scale, a difference between needleleaf (ENF) and deciduous broadleaf (DBF) forests is seen in both leaf gas exchange and stable isotope data, as has also been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

Consideration of decoupling between stomata and atmosphere (*sensu* Jarvis & McNaughton, 1986) did not help to explain this discrepancy (Fig. 7). We found that there was no difference in  $g_{1\text{-flux}}$  among forest types irrespective of whether the estimation of  $g_{1\text{-flux}}$  incorporated a decoupling factor. We found that median  $g_{1\text{-flux}}$  approached median  $g_{1\text{-leaf}}$  for needleleaf forests when decoupling was considered, and for broadleaf forests when it was not. This observation is supported by previous studies of scaling on single forests: a study on WUE in Scots pine found congruence between leaf and canopy WUE using a scaling approach incorporating decoupling (Launiainen *et al.*, 2011), whereas studies in broadleaf forests find congruence using approaches that do not consider decoupling (Barton *et al.*, 2012; Linderson *et al.*,

2012). However, it is generally thought that decoupling should be smallest in needleleaf canopies (Jarvis & McNaughton, 1986). This discrepancy clearly requires further investigation. Refining estimates of canopy stomatal and nonstomatal conductances from eddy flux data is one potential way forwards (e.g. Wehr *et al.*, 2017).

Leaf gas exchange also indicates a large difference in  $g_1$  between  $C_3$  and  $C_4$  species, as expected from their physiology. Although there was a clear difference in  $\Delta^{13}\text{C}$  between these two groups of species, we were unable to estimate  $g_{1\text{-isotope}}$  for the  $C_4$  species and hence unable to substantiate this difference in  $g_1$  at the leaf level using isotopic data. The issues involved in estimating  $C_i : C_a$  from  $\Delta^{13}\text{C}$  in  $C_4$  plants are discussed by Cernusak *et al.* (2013). A simple linear relationship was proposed by Henderson *et al.* (1992) but requires an estimate of bundle-sheath leakiness,  $\phi$ . Cernusak *et al.* (2013) suggest that  $\phi < 0.37$  under most environmental conditions. With this value of  $\phi$ , the linear relationship yields unrealistic values of  $C_i : C_a$  for much of the dataset, as the majority of measured values have  $\Delta^{13}\text{C} > 4.4\text{‰}$ . These data imply that either a value for  $\phi > 0.37$  is more commonly found in field conditions, or else that the simple linear relationship between  $\Delta^{13}\text{C}$  and  $C_i : C_a$  is inaccurate for leaf dry matter. Further research is needed to establish more widely applicable relationships between stable isotope data and WUE for  $C_4$  species.

Nonetheless, a difference in leaf-level  $g_1$  between  $C_3$  and  $C_4$  species is well documented in the literature (e.g. Morison & Gifford, 1983; Ghannoum *et al.*, 2011). Earlier studies synthesizing WUE from eddy covariance data did not explicitly address photosynthetic pathway (Law *et al.*, 2002; Beer *et al.*, 2009), and thus it was not known whether this fundamental leaf-level difference in  $g_1$  is reflected in canopy-scale gas exchange. Zhou *et al.* (2016) reported a difference in 'underlying WUE', an index similar to  $g_1$ , between  $C_3$  (corn) and  $C_4$  (soybean) crops at five Ameriflux sites. Similarly, we found a significant difference in  $g_{1\text{-flux}}$  between  $C_3$  and  $C_4$  crops that is consistent with the difference in  $g_{1\text{-leaf}}$  (Fig. 2). However, we did not find any evidence for lower

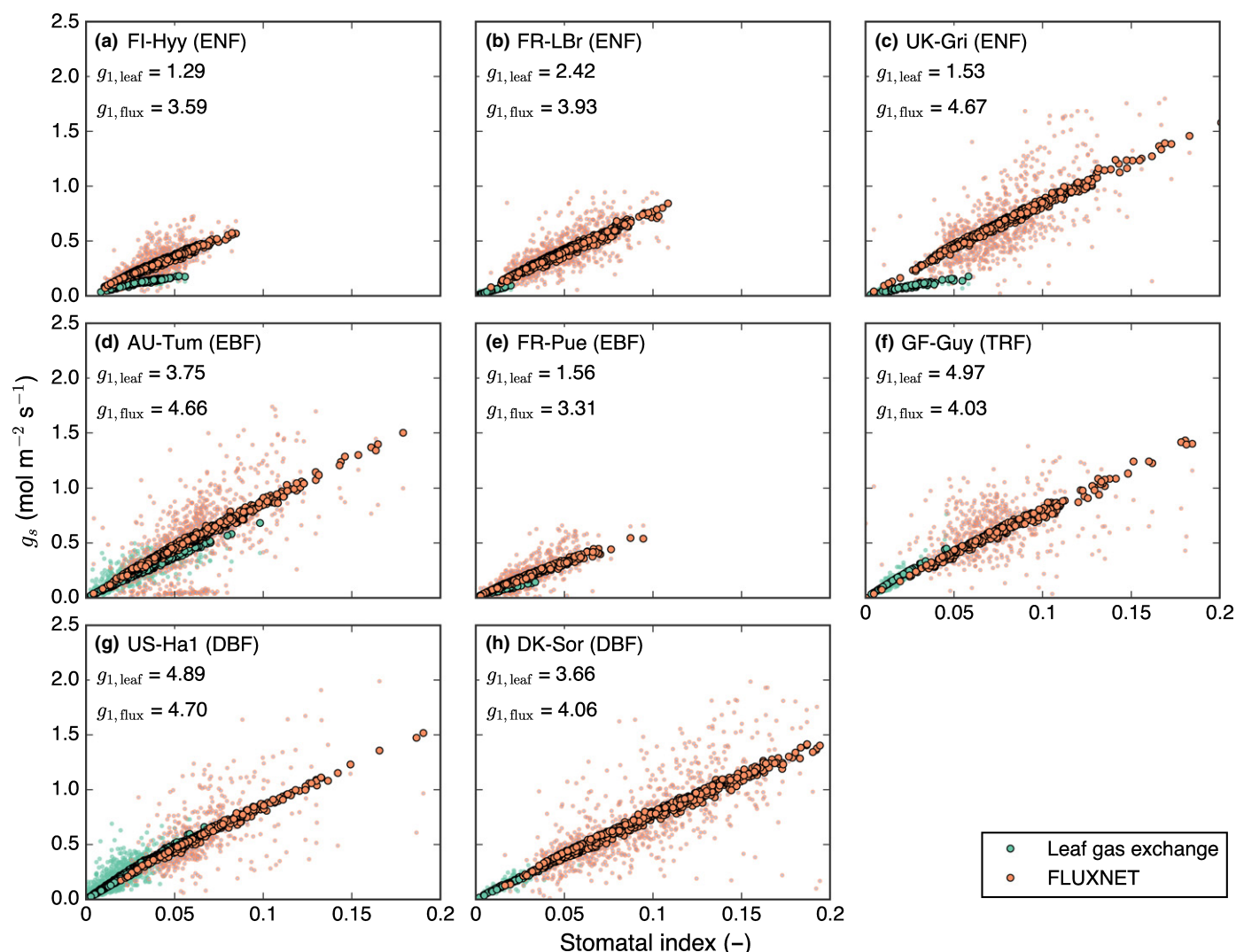


**Fig. 5** Estimated  $g_1$  values from leaf gas exchange, leaf isotope and FLUXNET data, shown as a function of latitude. Where several values were obtained at the same site (different species for leaf gas exchange and isotope, different years for FLUXNET), values have been averaged and standard error bars show variability. Plant functional types are defined as follows: ENF, evergreen needleleaf forest; EBF, evergreen broadleaf forest; DBF, deciduous broadleaf forest; TRF, tropical rainforest; SAV, savannah; SHB, shrub; C3G, C<sub>3</sub> grass; C4G, C<sub>4</sub> grass; C3C, C<sub>3</sub> crops; C4C, C<sub>4</sub> crops. Data shown have been clipped to a maximum  $g_1$  of 14.

$g_{1\text{-flux}}$  for grasslands with a C<sub>4</sub> component (Fig. 3). The difference in  $g_{1\text{-flux}}$  between C<sub>3</sub> and C<sub>4</sub> crops demonstrates that differences in  $g_{1\text{-leaf}}$  can scale to whole canopies, and that photosynthetic pathway must be considered when interpreting fluxes from crop canopies. The lack of an influence of photosynthetic pathway on

grassland  $g_{1\text{-flux}}$ , in contrast to crops, has several potential explanations. It is possible that there are significant evaporative fluxes from soil in grasslands that compensate for differences in transpiration between C<sub>3</sub> and C<sub>4</sub> vegetation. However, we also note that, owing to a lack of information at the site scale, we were





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

obliged to estimate  $C_4$  fraction in grasslands from a global dataset with relatively coarse resolution, suggesting that our characterization of  $C_4$  fraction may have been inaccurate. To correctly interpret fluxes from grasslands with a significant  $C_4$  component requires better quantification of vegetation  $C_3/C_4$  fraction at the site level. Furthermore, the estimated grassland  $C_4$  fraction did not exceed 0.4; data from grasslands known to have high  $C_4$  fraction are needed to test robustly for this effect. Finally, there is very high variability across site-years in  $g_{1-flux}$  estimates for  $C_3$ -only grasslands (Fig. 3), meaning our test lacks power; a better understanding of the reasons for this variability is needed to design fairer comparisons between  $C_3$ - and  $C_4$ -dominated grasslands.

### Relative $g_1$ values from different methods

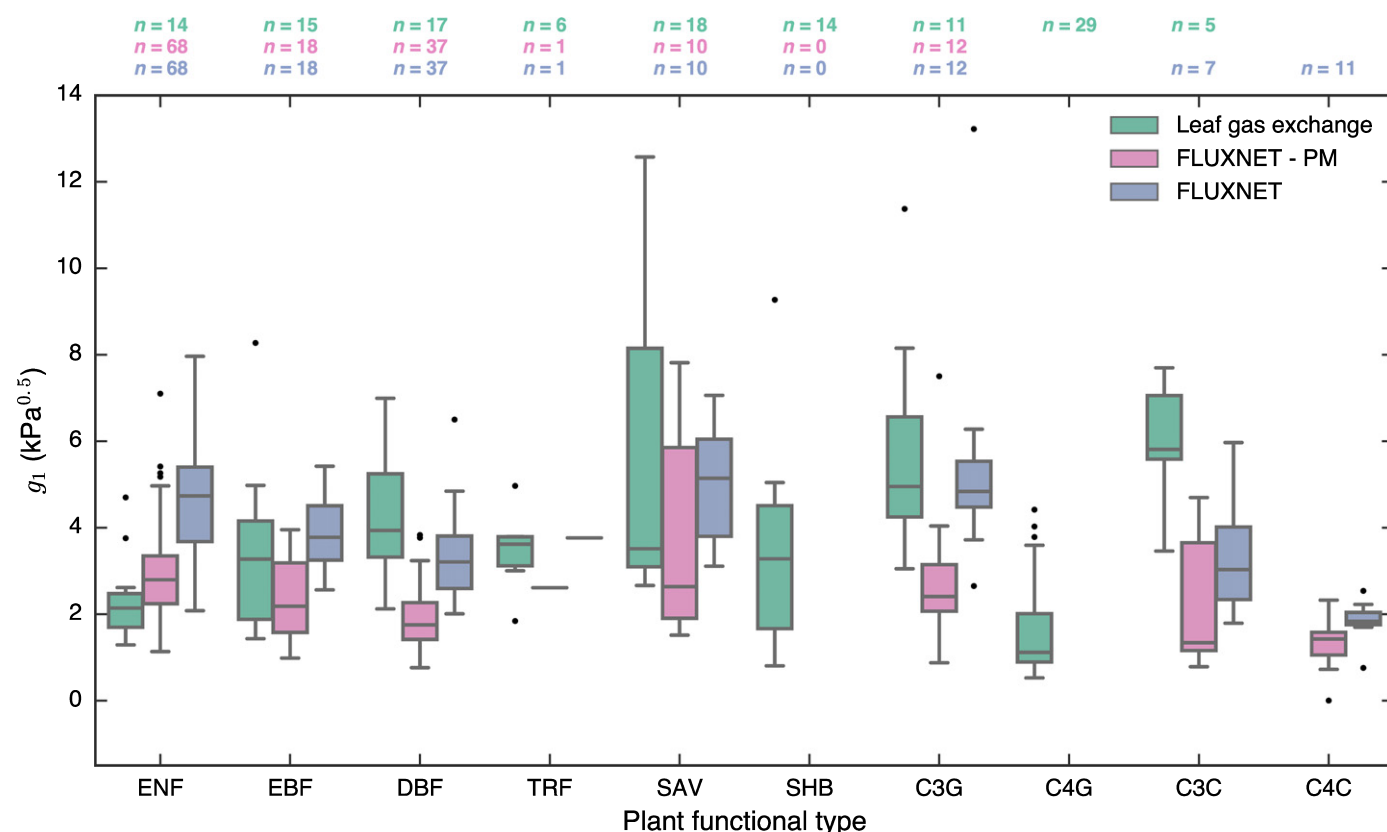
We predicted that  $g_{1-flux}$  values would exceed  $g_{1-leaf}$  values, because of additional water vapour loss from soil or canopy

evaporation (cf. Fig. 4). In contrast to our prediction, we found that once decoupling was taken into account, values of median  $g_{1-flux}$  were lower than values of  $g_{1-leaf}$  for several PFTs (Fig. 7). Significant within-canopy gradients in  $g_{1-leaf}$  can occur (e.g. Campamy *et al.*, 2016), but consideration of these gradients would also result in larger  $g_{1-flux}$  than canopy-top  $g_{1-leaf}$ . One potential explanation may be related to the use of GPP in the calculation of  $g_{1-flux}$ , rather than net photosynthesis (i.e. gross photosynthesis, less leaf respiration) as is used in the calculation of  $g_{1-leaf}$ . Recent work by Wehr *et al.* (2016) also suggests that the current method used to estimate GPP can overestimate daytime foliar respiration, which would tend to exaggerate the difference between GPP and net canopy photosynthesis. Further research is required to quantify the effect of including foliage respiration in estimation of  $g_{1-flux}$ , to determine if this mechanism is sufficient to account for low  $g_{1-flux}$  values.

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

FLUXNET Site ID	Latitude	Longitude	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, three campaigns (Nov-01, Feb-02, May-02)	Leuning <i>et al.</i> (2005)	Medlyn <i>et al.</i> (2007)
DK-Sor	55.49	12.10	5, 6, 7/1999	Diurnal spot measurements, upper canopy, 11 dates during Jun–Aug 99	Pilegaard <i>et al.</i> (2011)	Linderson <i>et al.</i> (2012)
FI-Hyy	61.85	24.29	5, 6, 7/2006	Automated shoot cuvette, upper canopy, continuous measurements, Jul-06	Vesala <i>et al.</i> (2005)	Kolari <i>et al.</i> (2007)
FR-LBr	44.72	−0.77	6, 7, 8/1997	Automated branch cuvette, upper canopy, continuous measurements, Sep-97	Berbigier <i>et al.</i> (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 <i>et al.</i> (2003)	Martin-StPaul <i>et al.</i> (2012)
GF-Guy	5.28	−52.93	6, 7, 8/2006	Light-saturated photosynthesis, upper canopy, Oct-10	Bonal <i>et al.</i> (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 <i>et al.</i> (2012)	Wingate <i>et al.</i> (2007)
US-Ha1	42.54	−72.17	6, 7, 8/1992	Diurnal spot measurements, upper canopy, monthly Jun–Sep 91/92	Urbanski <i>et al.</i> (2007)	Bassow & Bazzaz (1999)

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



**Fig. 7** Box and whisker plot (line, median; box, interquartile range) showing the estimated  $g_1$  values from leaf gas exchange, and FLUXNET data calculated using Eqn 4 to estimate canopy stomatal conductance (FLUXNET) or the Penman–Monteith equation (Eqn 5, FLUXNET-PM). The FLUXNET data are a subset of the data shown in Fig. 1 and include only those sites for which Eqn 5 could be applied. Whiskers extend to 1.5 times the interquartile range, with dots outside of the extent of the whiskers showing outlying values. Plant functional types (PFTs) are defined as follows: ENF, evergreen needleleaf forest; EBF, evergreen broadleaf forest; DBF, deciduous broadleaf forest; TRF, tropical rainforest; SAV, savannah; SHB, shrub; C3G, C<sub>3</sub> grass; C4G, C<sub>4</sub> grass; C3C, C<sub>3</sub> crops; C4C, C<sub>4</sub> crops.

We also predicted that  $g_{1\text{-isotope}}$  values would be lower than those of  $g_{1\text{-leaf}}$  as a result of mesophyll conductance ( $g_m$ ), which is neglected in the simplified isotopic theory used here to relate

leaf isotopic composition to  $C_i : C_a$  ratio (Evans *et al.*, 1986), although it has been suggested that the value of  $b$  used here (Eqn 2) should at least partially account for  $g_m$  effects (Seibt

*et al.*, 2008; Cernusak *et al.*, 2013). In support of our prediction, median values of  $g_{1\text{-isotope}}$  were lower than median values of  $g_{1\text{-leaf}}$  for all PFTs other than tropical rainforest (Fig. 2). The size of this effect should increase with increasing drawdown of  $\text{CO}_2$  from the intercellular airspace to the site of carboxylation; this drawdown is high in plants with low mesophyll conductance (typically ENF and EBF species; Niinemets *et al.*, 2009) and/or high photosynthetic rates. Nonetheless, we were surprised by the magnitude of the difference, which was substantial in most PFTs. Previous smaller-scale studies have found a good correspondence between leaf isotope and gas exchange measurements of  $C_i:C_a$  (e.g. Farquhar *et al.*, 1982; Orchard *et al.*, 2010). The size of this difference in our global data comparison suggests that use of the values of  $g_{1\text{-isotope}}$  to constrain large-scale models requires that  $g_m$  be taken into account. To do so, models will need a general quantitative knowledge of the drawdown of  $\text{CO}_2$  from the intercellular space to the mesophyll, which depends on both  $g_m$  and the photosynthetic rate (Evans *et al.*, 1986). As woody tissue is generally  $^{13}\text{C}$ -enriched compared with leaf tissue (Cernusak *et al.*, 2009), values of  $g_1$  estimated from tree ring stable isotopes would likely be lower still.

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

### Dataset biases

Each of the three datasets used in this study represents an enormous global scientific effort, and each is extremely valuable in advancing our understanding of the role of terrestrial vegetation in global carbon and water cycles. Nonetheless, each approach is subject to limitations. Leaf gas exchange measures are a direct and relatively accurate measure of the performance of a single leaf at a given point in time, but are inevitably restricted in sampling coverage. Measurements are often made only at the top of the canopy, for example, or only on a few days per season. There are some more extensive datasets in the Lin *et al.* (2015) database that were gathered through the use of *in situ* cuvettes (e.g. Kolari *et al.*, 2007; Op de Beeck *et al.*, 2010; Tarvainen *et al.*, 2013), but these remain the exception rather than the rule, and in any case cannot capture all potential sources of variation in the canopy. Stable isotope measures are more extensive (Fig. 2) but are less direct measures of gas exchange and, as our results show, may be influenced by other sources of isotopic discrimination. Other potential sources of error in interpreting stable isotope data

are the values assumed for long-term average daytime VPD, which are estimated from a global climate dataset (see the Materials and Methods section), and values assumed for source air  $\delta^{13}\text{C}$ . Eddy flux measurements have the advantage of measuring the behaviour of entire ecosystems, rather than individual leaves. However, these measurements are also subject to noise, and errors may be introduced in the estimation of GPP from measurements of net ecosystem  $\text{CO}_2$  exchange (Desai *et al.*, 2008). Furthermore, eddy flux data are known to suffer from an unresolved energy balance problem, in that the sum of latent and sensible heat fluxes is generally less than net radiation (Wilson *et al.*, 2002; Foken, 2008). The cause of this imbalance is not yet understood but may differ across sites. There are thus significant uncertainties associated with each of the three datasets. It is also important to be aware of potential bias introduced by different spatial coverage of the three datasets (Fig. 1). While we have been able to make some comparisons of different methodologies at specific sites (Fig. 6), more such comparisons – and comparisons with isotopic data – would be valuable (e.g. Monson *et al.*, 2010).

With global change accelerating, it is more important now than ever to make use of all available datasets to develop and constrain predictive models of vegetation function. Cross-comparison of methodologically independent datasets, as we have done here, is a crucial step forward. It highlights areas of inconsistency that should be high priorities for further research. It also quantifies the uncertainty associated with different measurement methods. Finally, our comparison indicates a need for understanding of potential biases when using any or all of these three datasets to constrain or validate ecosystem models that predict WUE.

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## Author contributions

B.E.M. and R.A.D. conceived and designed the study. B.E.M. led writing of paper. M.G.D.K. and Y-S.L. assembled and processed datasets, with assistance from J.K., R.A.D. and C.A.W. A.A., R.C., P.I., J-M.L., M-L.L., P.M., N.M-S. and L.W. assisted with interpretation of datasets. All authors contributed to writing of paper.

## References

- Barton CVM, Duursma RA, Medlyn BE, Ellsworth DS, Eamus D, Tissue DT, Adams MA, Conroy J, Crous KY, Liberloo M *et al.* 2012. Effects of elevated atmospheric CO<sub>2</sub> on instantaneous transpiration efficiency at leaf and canopy scales in *Eucalyptus saligna*. *Global Change Biology* 18: 585–595.
- Bassow S, Bazzaz F. 1999. Canopy photosynthesis study at Harvard Forest 1991–1992. Harvard Forest Data Archive: HF059.
- Beer C, Ciais P, Reichstein M, Baldocchi D, Law BE, Papale D, Soussana JF, Ammann C, Buchmann N, Frank D *et al.* 2009. Temporal and among-site variability of inherent water use efficiency at the ecosystem level. *Global Biogeochemical Cycles* 23: GB2018.
- Berbigier P, Bonnefond JM, Mellmann P. 2001. CO<sub>2</sub> and water vapour fluxes for 2 years above Euroflux forest site. *Agricultural & Forest Meteorology* 108: 183–197.
- Bonal D, Bosc A, Ponton S, Goret JY, Burban B, Gross P, Bonnefond JM, Elbers J, Longdoz B, Epron D *et al.* 2008. Impact of severe dry season on net ecosystem exchange in the Neotropical rainforest of French Guiana. *Global Change Biology* 14: 1917–1933.
- Bonan GB, Oleson KW, Fisher RA, Lasslop G, Reichstein M. 2012. Reconciling leaf physiological traits and canopy flux data: use of the TRY and FLUXNET databases in the Community Land Model version 4. *Journal of Geophysical Research-Biogeosciences* 117: G02026.
- Bonan GB, Williams M, Fisher RA, Oleson KW. 2014. Modeling stomatal conductance in the earth system: linking leaf water-use efficiency and water transport along the soil-plant-atmosphere continuum. *Geoscientific Model Development* 7: 2193–2222.
- Bosc A. 1999. *Etude expérimentale du fonctionnement hydrique et carboné des organes aériens du Pin maritime (Pinus pinaster Ait.)*. PhD thesis, Université Victor Segalen Bordeaux 2, France.
- Buchmann N, Brooks JR, Ehleringer JR. 2002. Predicting daytime carbon isotope ratios of atmospheric CO<sub>2</sub> within forest canopies. *Functional Ecology* 16: 49–57.
- Campany CE, Tjoelker MG, von Caemmerer S, Duursma RA. 2016. Coupled response of stomatal and mesophyll conductance to light enhances photosynthesis of shade leaves under sunflecks. *Plant, Cell & Environment* 39: 2762–2773.
- Cernusak LA, Tcherkez G, Keitel C, Cornwell WK, Santiago LS, Knohl A, Barbour MM, Williams DG, Reich PB, Ellsworth DS *et al.* 2009. Viewpoint: why are non-photosynthetic tissues generally <sup>13</sup>C enriched compared with leaves in C<sub>3</sub> plants? Review and synthesis of current hypotheses. *Functional Plant Biology* 36: 199–213.
- Cernusak LA, Ubierna N, Winter K, Holtum JAM, Marshall JD, Farquhar GD. 2013. Environmental and physiological determinants of carbon isotope discrimination in terrestrial plants. *New Phytologist* 200: 950–965.
- Clement RJ, Jarvis PG, Moncrieff JB. 2012. Carbon dioxide exchange of a Sitka spruce plantation in Scotland over five years. *Agricultural and Forest Meteorology* 153: 106–123.
- Cornwell WK, Wright IJ, Turner J, Maire V, Barbour M, Cernusak L, Dawson T, Ellsworth D, Farquhar G, Griffiths H *et al.* 2017. A global dataset of leaf Δ13C values. <https://doi.org/10.5281/zenodo.569501>
- Cowan IR, Farquhar GD. 1977. Stomatal function in relation to leaf metabolism and environment. In: Jennings DH, ed. *Integration of activity in the higher plant*. Cambridge, UK: Cambridge University Press, 471–505.
- Dekker SC, Groenendijk M, Booth BBB, Huntingford C, Cox PM. 2016. Spatial and temporal variations in plant water-use efficiency inferred from tree-ring, eddy covariance and atmospheric observations. *Earth System Dynamics* 7: 525–533.
- Desai AR, Richardson AD, Moffat AM, Kattge J, Hollinger DY, Barr A, Falge E, Noormets A, Papale D, Reichstein M *et al.* 2008. Cross-site evaluation of eddy covariance GPP and RE decomposition techniques. *Agricultural and Forest Meteorology* 148: 821–838.
- Diefendorf AF, Mueller KE, Wing SL, Koch PL, Freeman KH. 2010. Global patterns in leaf C-13 discrimination and implications for studies of past and future climate. *Proceedings of the National Academy of Sciences, USA* 107: 5738–5743.
- Evans JR, Sharkey TD, Berry JA, Farquhar GD. 1986. Carbon isotope discrimination measured concurrently with gas-exchange to investigate CO<sub>2</sub> diffusion in leaves of higher-plants. *Australian Journal of Plant Physiology* 13: 281–292.
- Farquhar GD, Ball MC, Von Caemmerer S, Roksandic Z. 1982. Effect of salinity and humidity on delta <sup>13</sup>C values of halophytes – evidence for diffusional isotope fractionation determined by the ratio of inter-cellular atmospheric partial-pressure of CO<sub>2</sub> under different environmental conditions. *Oecologia* 52: 121–124.
- Farquhar GD, Ehleringer JR, Hubick KT. 1989. Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* 40: 503–537.
- Foken T. 2008. The energy balance closure problem: an overview. *Ecological Applications* 18: 1351–1367.
- Frank DC, Poulter B, Saurer M, Esper J, Huntingford C, Helle G, Treydte K, Zimmermann NE, Schleser GH, Ahlstrom A *et al.* 2015. Water-use efficiency and transpiration across European forests during the Anthropocene. *Nature Climate Change* 5: 579–583.
- Ghannoum O, Evans JR, von Caemmerer S. 2011. Chapter 8 Nitrogen and water use efficiency of C<sub>4</sub> plants. In: Raghavendra AS, Sage RF, eds. *C<sub>4</sub> photosynthesis and related CO<sub>2</sub> concentrating mechanisms*. Dordrecht, the Netherlands: Springer Netherlands, 129–146.
- Groenendijk M, Dolman AJ, van der Molen MK, Leuning R, Arneeth A, Delpierre N, Gash JHC, Lindroth A, Richardson AD, Verbeeck H *et al.* 2011. Assessing parameter variability in a photosynthesis model within and between plant functional types using global Fluxnet eddy covariance data. *Agricultural and Forest Meteorology* 151: 22–38.
- Haverd V, Raupach MR, Briggs PR, Canadell JG, Isaac P, Pickett-Heaps C, Roxburgh SH, van Gorsel E, Rossel RAV, Wang Z. 2013. Multiple observation types reduce uncertainty in Australia's terrestrial carbon and water cycles. *Biogeosciences* 10: 2011–2040.
- Henderson SA, Von Caemmerer S, Farquhar GD. 1992. Short-term measurements of carbon isotope discrimination in several C<sub>4</sub> species. *Australian Journal of Plant Physiology* 19: 263–285.
- Henderson S, von Caemmerer S, Farquhar GD, Wade LJ, Hammer G. 1998. Correlation between carbon isotope discrimination and transpiration efficiency in lines of the C<sub>4</sub> species *Sorghum bicolor* in the glasshouse and the field. *Australian Journal of Plant Physiology* 25: 111–123.
- Jarvis PG, McNaughton KG. 1986. Stomatal control of transpiration – scaling up from leaf to region. *Advances in Ecological Research* 15: 1–49.
- Jung M, Henkel K, Herold M, Churkina G. 2006. Exploiting synergies of global land cover products for carbon cycle modeling. *Remote Sensing of Environment* 101: 534–553.
- Kala J, De Kauwe MG, Pitman AJ, Lorenz R, Medlyn BE, Wang YP, Lin YS, Abramowitz G. 2015. Implementation of an optimal stomatal conductance scheme in the Australian Community Climate Earth Systems Simulator (ACCESS1.3b). *Geoscientific Model Development* 8: 3877–3889.
- Kala J, De Kauwe MG, Pitman AJ, Medlyn BE, Wang Y-P, Lorenz R, Perkins-Kirkpatrick SE. 2016. Impact of the representation of stomatal conductance on model projections of heatwave intensity. *Scientific Reports* 6: 23418.



- Keenan TF, Hollinger DY, Bohrer G, Dragoni D, Munger JW, Schmid HP, Richardson AD. 2013. Increase in forest water-use efficiency as atmospheric carbon dioxide concentrations rise. *Nature* 499: 324–327.
- Kolari P, Lappalainen HK, Hanninen H, Hari P. 2007. Relationship between temperature and the seasonal course of photosynthesis in Scots pine at northern timberline and in southern boreal zone. *Tellus Series B-Chemical and Physical Meteorology* 59: 542–552.
- Launiainen S, Katul GG, Kolari P, Vesala T, Hari P. 2011. Empirical and optimal stomatal controls on leaf and ecosystem level CO<sub>2</sub> and H<sub>2</sub>O exchange rates. *Agricultural and Forest Meteorology* 151: 1672–1689.
- Law BE, Falge E, Gu L, Baldocchi DD, Bakwin P, Berbigier P, Davis K, Dolman AJ, Falk M, Fuentes JD *et al.* 2002. Environmental controls over carbon dioxide and water vapor exchange of terrestrial vegetation. *Agricultural and Forest Meteorology* 113: 97–120.
- Leuning R, Cleugh HA, Zegelin SJ, Hughes D. 2005. Carbon and water fluxes over a temperate Eucalyptus forest and a tropical wet/dry savanna in Australia: measurements and comparison with MODIS remote sensing estimates. *Agricultural and Forest Meteorology* 129: 151–173.
- Lin YS, Medlyn BE, Duursma RA, Prentice IC, Wang H, Baig S, Eamus D, de Dios VR, Mitchell P, Ellsworth DS *et al.* 2015. Optimal stomatal behaviour around the world. *Nature Climate Change* 5: 459–464.
- Linderson ML, Mikkelsen TN, Ibrom A, Lindroth A, Ro-Poulsen H, Pilegaard K. 2012. Up-scaling of water use efficiency from leaf to canopy as based on leaf gas exchange relationships and the modeled in-canopy light distribution. *Agricultural and Forest Meteorology* 152: 201–211.
- Lloyd J, Farquhar GD. 1994. C-13 discrimination during CO<sub>2</sub> assimilation by the terrestrial biosphere. *Oecologia* 99: 201–215.
- Medlyn BE, Duursma RA, Eamus D, Ellsworth DS, Prentice IC, Barton CVM, Crous KY, de Angelis P, Freeman M, Wingate L. 2011. Reconciling the optimal and empirical approaches to modelling stomatal conductance. *Global Change Biology* 17: 2134–2144.
- Medlyn BE, Pepper DA, O'Grady AP, Keith H. 2007. Linking leaf and tree water use with an individual-tree model. *Tree Physiology* 27: 1687–1699.
- Monson RK, Prater MR, Hu J, Burns SP, Sparks JP, Sparks KL, Scott-Denton LE. 2010. Tree species effects on ecosystem water-use efficiency in a high-elevation, subalpine forest. *Oecologia* 162: 491–504.
- Monteith JL. 1986. How do crops manipulate water-supply and demand. *Philosophical Transactions of the Royal Society a-Mathematical Physical and Engineering Sciences* 316: 245–259.
- Morison JIL, Gifford RM. 1983. Stomatal sensitivity to carbon-dioxide and humidity – a comparison of 2 C<sub>3</sub> and 2 C<sub>4</sub> grass species. *Plant Physiology* 71: 789–796.
- New M, Lister D, Hulme M, Makin I. 2002. A high-resolution data set of surface climate over global land areas. *Climate Research* 21: 1–25.
- Newville M, Stensitzki T, Allen DB, Ingargiola A. 2014. LMFIT: non-linear least-square minimization and curve-fitting for Python [Data set]. *Zenodo*. doi: 10.5281/zenodo.11813.
- Niinemets U, Diaz-Espejo A, Flexas J, Galmes J, Warren CR. 2009. Importance of mesophyll diffusion conductance in estimation of plant photosynthesis in the field. *Journal of Experimental Botany* 60: 2271–2282.
- Op de Beeck M, Low M, Deckmyn G, Ceulemans R. 2010. A comparison of photosynthesis-dependent stomatal models using twig cuvette field data for adult beech (*Fagus sylvatica* L.). *Agricultural and Forest Meteorology* 150: 531–540.
- Orchard KA, Cernusak LA, Hutley LB. 2010. Photosynthesis and water-use efficiency of seedlings from northern Australian monsoon forest, savanna and swamp habitats grown in a common garden. *Functional Plant Biology* 37: 1050–1060.
- Pilegaard K, Ibrom A, Courtney MS, Hummelshøj P, Jensen NO. 2011. Increasing net CO<sub>2</sub> uptake by a Danish beech forest during the period from 1996 to 2009. *Agricultural and Forest Meteorology* 151: 934–946.
- Rambal S, Ourcival JM, Joffre R, Mouillot F, Nouvellon Y, Reichstein M, Rocheteau A. 2003. Drought controls over conductance and assimilation of a Mediterranean evergreen ecosystem: scaling from leaf to canopy. *Global Change Biology* 9: 1813–1824.
- Saurer M, Spahni R, Frank DC, Joos F, Leuenberger M, Loader NJ, McCarroll D, Gagen M, Poulter B, Siegwolf RTW *et al.* 2014. Spatial variability and temporal trends in water-use efficiency of European forests. *Global Change Biology* 20: 3700–3712.
- Seibt U, Rajabi A, Griffiths H, Berry JA. 2008. Carbon isotopes and water use efficiency: sense and sensitivity. *Oecologia* 155: 441–454.
- Sinclair TR, Tanner CB, Bennett JM. 1984. Water-use efficiency in crop production. *BioScience* 34: 36–40.
- StPaul NKM, Limousin JM, Rodriguez-Calcerrada J, Ruffault J, Rambal S, Letts MG, Misson L. 2012. Photosynthetic sensitivity to drought varies among populations of *Quercus ilex* along a rainfall gradient. *Functional Plant Biology* 39: 25–37.
- Tarvainen L, Wallin G, Rantfors M, Uddling J. 2013. Weak vertical canopy gradients of photosynthetic capacities and stomatal responses in a fertile Norway spruce stand. *Oecologia* 173: 1179–1189.
- Thom AS. 1972. Momentum, mass and heat-exchange of vegetation. *Quarterly Journal of the Royal Meteorological Society* 98: 124–134.
- Urbanski S, Barford C, Wofsy S, Kucharik C, Pyle E, Budney J, McKain K, Fitzjarrald D, Czirkowsky M, Munger JW. 2007. Factors controlling CO<sub>2</sub> exchange on timescales from hourly to decadal at Harvard Forest. *Journal of Geophysical Research-Biogeosciences* 112: G02020.
- Vesala T, Suni T, Rannik U, Keronen P, Markkanen T, Sevanto S, Gronholm T, Smolander S, Kulmala M, Ilvesniemi H *et al.* 2005. Effect of thinning on surface fluxes in a boreal forest. *Global Biogeochemical Cycles* 19: GB2001, doi: 10.1029/2004GB002316.
- Wehr R, Commene R, Munger JW, McManus JB, Nelson DD, Zahniser MS, Saleska SR, Wofsy SC. 2017. Dynamics of canopy stomatal conductance, transpiration, and evaporation in a temperate deciduous forest, validated by carbonyl sulfide uptake. *Biogeosciences* 14: 389–401.
- Wehr R, Munger JW, McManus JB, Nelson DD, Zahniser MS, Davidson EA, Wofsy SC, Saleska SR. 2016. Seasonality of temperate forest photosynthesis and daytime respiration. *Nature* 534: 680–683.
- Wilson K, Goldstein A, Falge E, Aubinet M, Baldocchi D, Berbigier P, Bernhofer C, Ceulemans R, Dolman H, Field C *et al.* 2002. Energy balance closure at FLUXNET sites. *Agricultural and Forest Meteorology* 113: 223–243.
- Wingate L, Seibt U, Moncrieff JB, Jarvis PG, Lloyd J. 2007. Variations in <sup>13</sup>C discrimination during CO<sub>2</sub> exchange by *Picea sitchensis* branches in the field. *Plant, Cell & Environment* 30: 600–616.
- Zhou S, Yu B, Huang YF, Wang GQ. 2015. Daily underlying water use efficiency for AmeriFlux sites. *Journal of Geophysical Research-Biogeosciences* 120: 887–902.
- Zhou S, Yu BF, Zhang Y, Huang YF, Wang GQ. 2016. Partitioning evapotranspiration based on the concept of underlying water use efficiency. *Water Resources Research* 52: 1160–1175.