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Observed and modelled historical trends in the water-use efficiency of plants and ecosystems

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Abstract

Plant water-use efficiency (WUE, the carbon gained through photosynthesis per unit of water lost through transpiration) is a tracer of the plant physiological controls on the exchange of water and carbon dioxide between terrestrial ecosystems and the atmosphere. At the leaf level, rising CO₂ concentrations tend to increase carbon uptake (in the absence of other limitations) and to reduce stomatal conductance, both effects leading to an increase in leaf WUE. At the ecosystem level, indirect effects (e.g. increased leaf area index, soil water savings) may amplify or dampen the direct effect of CO₂. Thus, the extent to which changes in leaf WUE translate to changes at the ecosystem scale remains unclear. The differences in the magnitude of increase in leaf versus ecosystem WUE as reported by several studies are much larger than would be expected with current understanding of tree physiology and scaling, indicating unresolved issues. Moreover, current vegetation models produce inconsistent and often unrealistic magnitudes and patterns of variability in leaf and ecosystem WUE, calling for a better assessment of the underlying approaches. Here, we review the causes of variations in observed and modelled historical trends in WUE over the continuum of scales from leaf to ecosystem, including methodological issues, with the aim of elucidating the reasons for discrepancies observed within and across spatial scales. We emphasize that even though physiological responses to changing environmental drivers should be interpreted differently depending on the observational scale, there are large uncertainties in each data set which are often underestimated. Assumptions

made by the vegetation models about the main processes influencing WUE strongly impact the modelled historical trends. We provide recommendations for improving long-term observation-based estimates of WUE that will better inform the representation of WUE in vegetation models.

KEYWORDS: carbon isotopic discrimination, eddy-covariance flux, spatial scales, stomatal conductance, trends in water-use efficiency, vegetation modelling

1 INTRODUCTION

Plant water-use efficiency (WUE)—the ratio of carbon uptake through photosynthesis per unit water loss through transpiration—is an indicator of the relative rate of exchange of CO₂ and water between the vegetation and the atmosphere (Eamus, 1991; Morison, 1985; Saxe, Ellsworth, & Heath, 1998). In the absence of other limitations and when plant acclimation is limited, rising atmospheric CO₂ concentrations (c_a) tend to increase carbon assimilation of C₃ plants (Ainsworth & Long, 2005; Franks et al., 2013) and to reduce stomatal conductance (g_s) and therefore transpiration (T_r) (Jarvis, 1976; Mansfield, 1967), leading to an increase in leaf WUE on relatively short timescales (Ainsworth & Rogers, 2007). Changes in soil moisture and/or atmospheric water demand (i.e. leaf vapour pressure deficit; D) also contribute to modulating WUE via their effects on g_s (Beer et al., 2009; Linares & Camarero, 2012). The ecosystem responses to elevated c_a and changes in water availability remain more uncertain. Although physical and physiological processes underlying carbon and water fluxes occur over a continuum of scales, indirect effects and feedbacks at the whole ecosystem scale may amplify or dampen the direct leaf-level response to c_a (Fatichi, Leuzinger, Paschalis, Langley, & Donnellan, 2016). These indirect effects include changes in leaf area index and canopy structure (Gerten, Rost, Bloh, & Lucht, 2008) and atmospheric boundary-layer feedbacks (Field, Jackson, & Mooney, 1995; De Kauwe et al., 2013). Thus, scaling up the terrestrial carbon and water response to environmental stimuli from leaf to canopy and ecosystem is a key uncertainty (De Kauwe, Medlyn, Knauer, & Williams, 2017; Way, Oren, & Kroner, 2015), and physiological responses to changing environmental drivers should be interpreted differently depending on the observational scale (Yi et al., 2018).

There are different ways of quantifying temporal changes in WUE based on observations, depending on the scale of investigation (leaf level vs. ecosystem scale), the temporal resolution (half-hourly to yearly), the time length (interannual to multicentennial scales), the type of data (stable carbon isotopes in plant materials or eddy-covariance flux measurements), and the specific definition adopted for WUE. As a result, data-based estimates of WUE are not easily comparable and often appear to disagree with one another (e.g. Frank et al., 2015; Keenan et al., 2013; Keller et al., 2017), obscuring the interpretation of the results. Even though inherent differences between plant functional types (PFTs) have been reported

(Brodribb, McAdam, Jordan, & Feild, 2009; Lin et al., 2015), and though varying site conditions may induce different physiological responses, the large variation in WUE trends between tree-ring-based estimates and those from eddy-covariance measurements reported in the literature is unexpected (Medlyn & De Kauwe, 2013) and at odds with current understanding of scaling. Our goal here is to examine recent estimates of long-term changes in WUE from the leaf to the ecosystem scale in order to better understand the apparent differences observed between approaches. Several studies have attempted to reconcile estimates of WUE across scales (e.g. Guerrieri, Lepine, Asbjornsen, Xiao, & Ollinger, 2016; Medlyn et al., 2017; Scartazza et al., 2014; Yi et al., 2018), but, to our knowledge, no study has yet compared trend estimates from different scales; most research has addressed a single scale of investigation. There is still a challenge in bridging observational data sets from multiple spatial and temporal scales to capture long-term changes in WUE under a changing climate.

This paper reviews the causes of variations in observed and modelled historical trends in WUE across scales, emphasizing that differences may not only be driven by the varying impacts of environmental factors on WUE at the different observational scales, but may also be a consequence of the large uncertainties related to the data set or the model considered. We initially compare the various definitions and measurement techniques applied to infer long-term changes in WUE. We then present a synthesis and interpretation of the discrepancies observed within and across scales, including methodological uncertainties. Lastly, we discuss the performance of vegetation models to reproduce observed changes in WUE at the different scales of investigation, and suggest ways in which both numerical modelling and the interpretation of the data could be put on a firmer basis. Our aim is to provide recommendations for improving observation-based estimates of WUE, which will better inform and therefore improve the representation of WUE in vegetation models.

2 ESTIMATING HISTORICAL TRENDS IN WATER-USE EFFICIENCY AT THE LEAF versus ECOSYSTEM SCALE

We focus attention on stable carbon isotopes in tree rings and eddy-covariance flux measurements. We do not include leaf gas exchange data because, while they are commonly used to estimate instantaneous leaf-level water and carbon exchanges (Long & Bernacchi, 2003; Wingate, Seibt, Moncrieff, Jarvis, & Lloyd, 2007), they are not useful for estimating long-term trends because of the practical impossibility of adequate long-term sampling. We do not include estimates of WUE based on remotely sensed vegetation greenness and carbon and water fluxes (Hobeichi, Abramowitz, Evans, & Ukkola, 2018; Parazoo et al., 2014), because none of the data sets used are solely driven by remotely sensed observations—they rely upon models and invoke substantial assumptions. Nevertheless, given the increasing interest in using remote sensing for investigating spatial changes in forest carbon

and water balance, we have addressed them in the Supporting Information (Text S1).

Here, we describe the equations that have been applied to derive the general trends in WUE expected with environmental changes at the different scales of investigation and the measurements used to estimate them (Table 1). We acknowledge that other formulations of WUE exist (e.g. Farquhar & Richards, 1984; Medlyn et al., 2017; Zhou, Yu, Huang, & Wang, 2014), but here we focus on the most commonly used to assess long-term trends.

TABLE 1 Commonly used formulations of WUE, and measurements used for inferring trends

Form of WUE	Spatial scale	Short name	Equation	Temporal scale	Measurements used ^a	Data processing	Open-access products ^b
"intrinsic"	Leaf	iWUE ^{leaf}	4	Seasonal to yearly	Stable carbon isotopic composition of bulk wood or α cellulose in tree rings ($\delta^{13}C_{TR}$) + atmospheric $\delta^{13}CO_2$	Calculation of $\Delta^{13}C$; corrections for postphotosynthetic fractionations (d) and intersite variability (ϵ)	ITRDB + Graven et al. (2017)
"instantaneous"	Ecosystem	WUE ^{eco}	5	Half-hourly or hourly	Eddy-covariance fluxes (LE, H, NEE, GPP) + R_n , T_{air} , VPD, c_a	Flux partitioning and data postprocessing (see Text S2)	FLUXNET-2015 Tier 1
"inherent"	Ecosystem	iWUE ^{eco}	8				

^aLatent heat flux (LE), sensible flux (H), net ecosystem exchange of CO₂ between the ecosystem and the atmosphere (NEE), gross primary production (GPP), air temperature (T_{air}), vapour pressure deficit (VPD), net radiation (R_n), precipitation (P).

^bInternational Tree-Ring Data Bank (ITRDB; <https://www.ncdc.noaa.gov/dataaccess/paleoclimatology-data/datasets/tree-ring>), eddy-covariance flux towers network (FLUXNET; <http://fluxnet.fluxdata.org/data>).

2.1 Leaf-level estimates

At the leaf level, the "intrinsic" WUE (iWUE^{leaf}, $\mu\text{mol}/\text{mol}$) is defined as the ratio of photosynthetic assimilation rate (A , $\mu\text{mol m}^{-2} \text{s}^{-1}$) to stomatal conductance for H₂O diffusion (g_{sw} , $\text{mol m}^{-2} \text{s}^{-1}$) as follows (Ehleringer, Hall, & Farquhar, 1993):

$$iWUE^{leaf} = \frac{A}{g_{sw}} = \frac{g_{sc}}{g_{sw}} (c_a - c_i) = \frac{g_{sc}}{1.6g_{sc}} c_a \left(1 - \frac{c_i}{c_a}\right) = \frac{c_a}{1.6} \left(1 - \frac{c_i}{c_a}\right) \quad (1)$$

where g_{sc} is the stomatal conductance for CO₂ diffusion ($\text{mol m}^{-2} \text{s}^{-1}$) and c_i and c_a are the leaf-intercellular and ambient CO₂ concentrations, respectively ($\mu\text{mol mol}^{-1}$). The factor 1.6 arises because H₂O diffuses through air more rapidly than CO₂. Note that although g_{sc} has been eliminated from the right-hand side of Equation (1), stomatal regulation of iWUE^{leaf} is implicit, as it controls the ratio of c_i to c_a . Equation (1) predicts that iWUE^{leaf} should increase with c_a and vary if c_i/c_a varies. The only unknown in this equation is c_i/c_a , which can be estimated using stable carbon isotopic composition of plant materials. During CO₂ diffusion through the stomata and photosynthesis, plants assimilate more rapidly the lighter ¹²CO₂ molecules compared to the heavier ¹³CO₂ ones (Park & Epstein, 1960), resulting in a quantifiable discrimination against ¹³C ($\Delta^{13}C$). The calculation of $\Delta^{13}C$ (‰) requires only knowledge of $\delta^{13}C$ of the plant material ($\delta^{13}C_p$, i.e. the ratio of ¹³C to ¹²C of the wood component compared to an internationally accepted standard material) and that of the ambient air ($\delta^{13}C_{atm}$):

$$\Delta^{13}\text{C} = \frac{\delta^{13}\text{C}_{\text{atm}} - \delta^{13}\text{C}_p}{1 + \delta^{13}\text{C}_p/1000} \quad (2)$$

In C_3 plants, $\Delta^{13}\text{C}$ depends principally on the gradient of concentration of CO_2 from the external atmosphere to the intercellular spaces, and therefore on c_i/c_a . Thus, estimating c_i/c_a from $\delta^{13}\text{C}$ measured in tree rings can provide evidence for changes in $i\text{WUE}^{\text{leaf}}$ (Table 1).

By far the most commonly used formula to estimate the c_i/c_a ratio from $\Delta^{13}\text{C}$ is the following simple expression (Farquhar, O'Leary, & Berry, 1982):

$$\Delta^{13}\text{C} = a + (\bar{b} - a) \cdot \left(\frac{c_i}{c_a} \right) \quad (3)$$

where a represents the fractionation due to CO_2 diffusion in air (4.4‰; Craig, 1953) and \bar{b} is the apparent net fractionation by Rubisco during carboxylation (27‰; Farquhar, Ball, Ball, Caemmerer, & Roksandic, 1982). Equation (3) is convenient because it implies that any change in $\Delta^{13}\text{C}$ must be attributed to a change in c_i/c_a . It is a good predictor of variations in c_i/c_a values independently estimated from gas exchange measurements (Cernusak et al., 2013). However, this equation is only strictly applicable for the first products of photosynthesis—it does not consider downstream postphotosynthetic fractionations when photosynthates are used to construct the various components of wood (Cernusak, Winter, & Turner, 2009; Gessler et al., 2009). Measurements of compound-specific fractionation between leaf/needle organic matter and wood components (Gessler et al., 2014; Rinne et al., 2015) can be used to estimate these postphotosynthetic fractionation processes and quantify uncertainties associated with the estimation of $i\text{WUE}^{\text{leaf}}$ using tree rings. Equations (1) and (3) may thus be combined to give $i\text{WUE}^{\text{leaf}}$ in terms of $\Delta^{13}\text{C}$ adjusted for postphotosynthetic fractionation processes as in Frank et al. (2015):

$$i\text{WUE}^{\text{leaf}} = \frac{c_a}{1.6} \left(1 - \frac{\left(\frac{\delta^{13}\text{C}_{\text{atm}} - (\delta^{13}\text{C}_p - d)}{1 + (\delta^{13}\text{C}_p - d)/1000} \right) - a}{(\bar{b} - a)} \right) + \varepsilon \quad (4)$$

where d quantifies the sum of discriminations beyond those associated with the production of the primary photosynthetic assimilates (2.1 ± 1.2 ‰ between leaf organic matter and α -cellulose) and ε represents variability among trees within a site (0 ± 0.8 ‰; Frank et al., 2015).

Equation (4) provides a measure for the relative water loss per unit carbon acquired at the leaf level. However, $i\text{WUE}^{\text{leaf}}$ may be better considered as potential rather than actual WUE as this formulation does not directly account for changes in evaporative demand and respiratory losses (Farquhar, Ehleringer, & Hubick, 1989; Seibt, Rajabi, Griffiths, & Berry, 2008).

Nevertheless, even though $iWUE^{\text{leaf}}$ excludes the direct influence of D , it is still dependent on D , through changes in g_s (Cowan & Farquhar, 1977). So comparisons of $iWUE^{\text{leaf}}$ should ideally be restricted to situations with similar D (Franks et al., 2013).

2.2 Ecosystem-scale estimates

At the ecosystem scale, the simplest definition of ecosystem WUE is WUE^{eco} ($\text{gC kg H}_2\text{O}^{-1}$), defined as (Law et al., 2002):

$$WUE^{\text{eco}} = \frac{GPP}{ET} \quad (5)$$

where GPP is gross primary production ($\text{gC m}^{-2} \text{s}^{-1}$) and ET is evapotranspiration ($\text{kg H}_2\text{O m}^{-2} \text{s}^{-1}$), which includes contributions from rainfall interception (I_n) (water that is evaporated from the canopy surface), bare-ground evaporation (E_v), and T_r . This measure is difficult to interpret, however, given that it includes evaporative components unrelated to physiological processes. The ecosystem-level equivalent of leaf-level $iWUE$ is $iWUE^{\text{eco}}$, defined as:

$$iWUE^{\text{eco}} = \frac{GPP}{G_s} \quad (6)$$

where G_s is the bulk surface conductance of the ecosystem (m/s or $\text{mol m}^{-2} \text{s}^{-1}$) calculated by inverting the Penman–Monteith equation (Monteith, 1965):

$$G_s = \frac{G_a \cdot \gamma \cdot LE}{s \cdot (R_n - G) - LE \cdot (s + \gamma) + G_a \cdot \rho \cdot c_p \cdot D} \quad (7)$$

where G_a is aerodynamic conductance (m/s), R_n is net radiation (W/m^2), G is the ground heat flux (W/m^2), LE is the latent heat flux (W/m^2), s is the slope of the saturated vapour pressure with air temperature (Pa/K), ρ is the density of air (kg/m^3), c_p is the specific heat of air ($\text{J kg}^{-1} \text{K}^{-1}$), and γ is the psychrometric constant (Pa/K).

As G_s can only be estimated indirectly, the alternative “inherent” ecosystem WUE, or $iWUE^{\text{eco}}$, has been proposed following Beer et al. (2009), assuming equal temperatures of leaf and atmosphere and full atmosphere–canopy coupling (i.e. infinite G_a ; McNaughton & Black, 1973):

$$iWUE^{\text{eco}} = \frac{GPP}{ET} \cdot \frac{VPD}{P_{\text{atm}}} \quad (8)$$

where P_{atm} is atmospheric pressure (Pa). Note that the meteorological (above-canopy) vapour pressure deficit (VPD) in Equation (8) is not the same quantity as the D that appears in Equation (7). Nonetheless, as GPP is the integrated value of A over the whole canopy, and as ET is assumed to be dominated by T_r in all but the sparsest canopies ($T_r \gg I_n + E_v$ when 1–2 days after rains are ignored to minimize the other contributions to the total ET flux), WUE^{eco} , $iWUE^{\text{eco}}$, and $iWUE^{\text{eco}}$ are all expected a priori to change in

response to environmental variations in a similar fashion to $iWUE^{leaf}$, when not considering interactions or feedbacks.

Most variables needed to infer ecosystem WUE from Equations (5–8–5–8) can be estimated from eddy-covariance flux measurements above vegetation canopies. Flux towers measure the net ecosystem exchange of CO_2 (NEE , $mol\ m^{-2}\ s^{-1}$), meteorological variables (including VPD and air temperature, T_{air}), and the sensible (H) and latent heat (LE) fluxes between ecosystems and the atmosphere (W/m^2). NEE is the difference between the total ecosystem respiration (R_{eco}), which includes heterotrophic and autotrophic respiration (R_H and R_A), and GPP. Various flux partitioning algorithms are commonly applied to infer GPP and R_{eco} from measured NEE (Lasslop et al., 2010; Reichstein, Stoy, Desai, Lasslop, & Richardson, 2012; see Text S2 for further details). ET is derived from LE by:

$$ET = \frac{LE}{\lambda_v} \quad (9)$$

where λ_v is the latent heat of vaporization, that is the amount of energy required to evaporate 1 kg liquid water (kJ/kg), which depends slightly on T_{air} (Stull, 1988).

Applying Equation (6) to estimate ecosystem WUE trends is recommended as it is designed to both minimize the influence of nonstomatal water fluxes (e.g. soil and canopy evaporation) and account for changes in atmospheric demand. New tools are now available to facilitate the derivation of G_a and G_s from the eddy-covariance flux observations (Knauer, El-Madany, El-Madany, Zaehle, & Migliavacca, 2018)—but all methods to date necessitate a “big-leaf” assumption in the inversion of G_s , which introduces additional assumptions.

3 TRENDS REPORTED IN THE LITERATURE FROM OBSERVATIONS

Over the past 150 years, c_a has increased by 41% (Le Quéré et al., 2018), suggesting that WUE should have increased proportionally to c_a —although simultaneous changes in climate (especially increasing evaporative water demand with rising temperature) could have modified this trend (Donohue, Roderick, McVicar, & Farquhar, 2013). Consistent with this expectation, most empirical studies have shown increasing trends in both leaf- and ecosystem-level WUE concurrently with the c_a rise over the 20th and 21st centuries. However, different magnitudes of WUE increase have been documented in the literature.

Leaf-level studies using stable carbon isotopes in tree rings, employing Equation (4), have indicated an increase of $iWUE^{leaf}$ of $0.2 \pm 0.1\% \text{ year}^{-1}$ over the 20th century (e.g. Frank et al., 2015; Keller et al., 2017; Peñuelas, Canadell, & Ogaya, 2011; Saurer et al., 2014; Silva & Anand, 2013), approximately proportional to the recent increase in c_a (Figure 1b). Some of these studies have shown a weakening $iWUE^{leaf}$ response to c_a towards the end of the 20th century in regions where increases both in temperature and

in D may have offset the increase in $iWUE^{leaf}$ due to increasing c_a (Gagen et al., 2011; Waterhouse et al., 2004). In contrast, ecosystem studies using eddy-covariance measurements have reported increases up to 2.3% year⁻¹ over the period 1995–2010 (Keenan et al., 2013), albeit with a slight decrease in magnitude over recent years (i.e. $1.3 \pm 0.1\%$ year⁻¹ over 1991–2014; Mastrotheodoros et al., 2017; Wang, Chen, Wu, & Bai, 2018). These studies reported a much stronger increase in ecosystem WUE relative to c_a rise than that shown by tree-ring studies (Figure 1b). Note, however, that changes in ecosystem-level WUE based on eddy-covariance data were estimated using different formulations, that is Equation (8) (Keenan et al., 2013; Mastrotheodoros et al., 2017) versus Equation (6) (Wang et al., 2018), which may have led to some differences in the estimated magnitude of trends (Figure 1b).

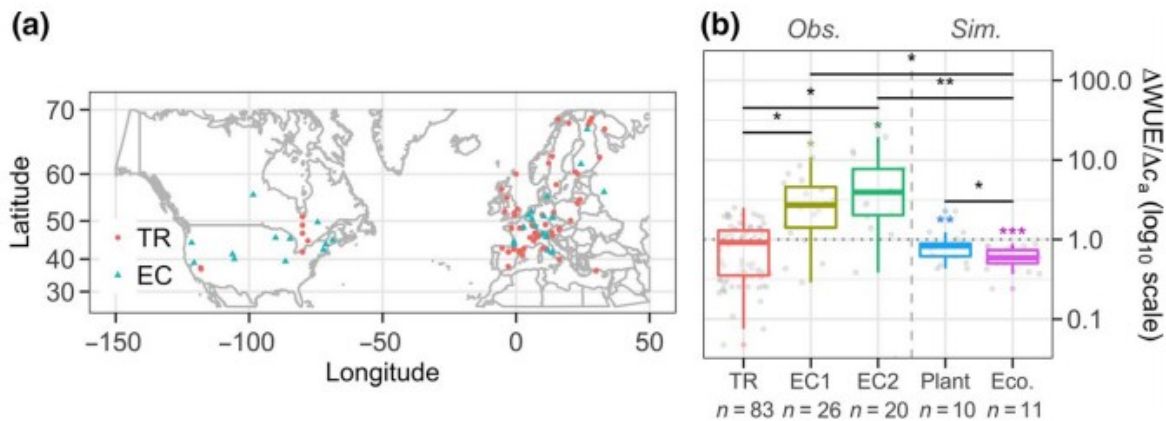


Figure 1. (a) Geographical locations of the selected 83 tree-ring (TR) sites and 34 eddy-covariance (EC) flux sites with estimates of water-use efficiency change (ΔWUE) available in the literature (see Tables S1–S3). (b) ΔWUE inferred from TR and EC observations over the historical period (Tables S1–S3) or from vegetation model simulations depicted in the literature (Table S4) compared to change in atmospheric CO₂ concentrations (Δc_a) over the same period. EC1-based and EC2-based ecosystem WUE estimates are for $iWUE^{eco}$ (Equation (6); Table S2) and $iWUE^{eco}$ (Equation (8); Table S3), respectively. Δc_a is calculated using c_a values derived from a merged product of atmospheric CO₂ record based on ice core data and in situ direct measurements (http://scrippsco2.ucsd.edu/data/atmospheric_co2/icecore_merged_products; last update on 12 December 2018). Results are presented in a log scale. The grey horizontal dotted line in (b) describes a 1:1 relationship between ΔWUE and Δc_a , equivalent to a 1:1 relationship between Δc_i and Δc_a . Significant differences between $\Delta WUE/\Delta c_a$ estimates and the 1:1 line (asterisks in colour) and between the different groups of $\Delta WUE/\Delta c_a$ (black lines and asterisks) are also indicated (Student's t test or Wilcoxon test; $p < 0.05$, *, $p < 0.01$, **, and $p < 0.001$, ***)

The reasons for the large differences in the apparent magnitude of WUE change between scales are debated. A key issue is that the strong physiological response at the ecosystem scale, implied by eddy-covariance analyses, would produce substantial changes in surface hydrology (via reduced ET) at the continental scale, but such changes appear to be inconsistent with observed large-scale trends in continental runoff, ET, and the seasonal amplitude of atmospheric CO₂ (Knauer et al., 2017). In the next sections, we discuss the potential strategies of leaf gas exchange that plants

may have followed with rising c_a , via changes in c_i , to explain the large differences in trends between leaf and ecosystem estimates. We also investigate the different methodological approaches applied for assessing trends that may have contributed to these differences.

3.1 Long-term leaf gas exchange strategies: implications for trends in water-use efficiency

The primary effects on leaves of rising c_a have been well documented in experimental studies of individual plant species grown in controlled environments (Norby, Wullschleger, Gunderson, Johnson, & Ceulemans, 1999; Saxe et al., 1998). However, while some studies have shown that an increase of c_a would lead to an increase of the difference in CO_2 concentrations across the stomata to maintain a constant c_i/c_a (Masle, Farquhar, & Gifford, 1990; Polley, Johnson, Marinot, & Mayeux, 1993; Wong, Cowan, & Farquhar, 1979), others suggested that c_i may be held constant across a range of c_a (Ehleringer & Cerling, 1995; Francey & Farquhar, 1982), or even that c_i may increase in a similar magnitude as c_a , holding $c_a - c_i$ constant (Marshall & Monserud, 1996). Based on these controlled experimental studies and as a general framework for benchmarking trends in WUE, Saurer, Siegwolf, and Schweingruber (2004) proposed three hypothetical scenarios reflecting specific leaf gas exchange response to changing c_a in which leaves maintain either: (i) constant c_i , (ii) constant $c_a - c_i$, or (iii) constant c_i/c_a . Following Equations (1) and (2), and assuming no change in D , the first scenario (i) implies a strong increase of $i\text{WUE}^{\text{leaf}}$ with decreasing c_i/c_a , augmenting the effect of increasing c_a ; the second scenario (ii) implies constant $i\text{WUE}^{\text{leaf}}$ with increasing c_i/c_a , counteracting the effect of increasing c_a ; and the third scenario (iii) is intermediate, with $i\text{WUE}^{\text{leaf}}$ increasing in proportion to c_a .

With one exception (Battipaglia et al., 2013), free-air carbon dioxide enrichment (FACE) experiments studying the effects of elevated c_a on plants and ecosystems under natural, open-air conditions have shown responses broadly consistent with the third strategy, that is constant c_i/c_a (Ainsworth & Long, 2005; Gimeno et al., 2016; De Kauwe et al., 2013), giving rise to the expectation that plants growing in normal conditions should behave accordingly. Most tree-ring studies in natural environments have yielded results consistent with this strategy (Frank et al., 2015; Keller et al., 2017; Peñuelas et al., 2011; Saurer et al., 2014). However, there is also empirical support for plants following scenario (i) with constant c_i (Dorado Liñán et al., 2011) or scenario (ii) where c_i increases at the same rate as c_a (McCarroll et al., 2009; Treydte et al., 2009). Other tree-ring studies moreover have indicated intermediate situations between the constant c_i and constant c_i/c_a scenarios, implying a more than expected increase in $i\text{WUE}^{\text{leaf}}$, or between the constant $c_a - c_i$ and constant c_i/c_a scenarios, associated with relatively moderate increase in $i\text{WUE}^{\text{leaf}}$ (Andreu-Hayles et al., 2011; Lavergne et al., 2017; Leonardi et al., 2012; Urrutia-Jalabert, Malhi, Barichivich, & Lara, 2015). Several hypotheses to explain the apparent differences in leaf gas

exchange strategies between studies have been proposed, including genetically determined variations in leaf architecture, gas exchange characteristics, and photosynthetic capacities between species. On the one hand, the greater leaf hydraulic conductance in angiosperms than in gymnosperms (Sperry, Hacke, & Pittermann, 2006) may induce higher photosynthetic capacities and stronger stomatal responsiveness to rising c_a for the former (Brodribb et al., 2005, 2009). On the other hand, reductions in g_s are likely to alter photosynthesis less and generate a stronger response of c_i/c_a and $iWUE^{leaf}$ in species with more rigid leaf architecture, including most gymnosperms (Niinemets, Díaz-Espejo, Flexas, Galmés, & Warren, 2009; Niinemets, Flexas, & Peñuelas, 2011). The combined impacts of these phylogenetic differences on changes in c_i/c_a and $iWUE^{leaf}$ are largely unknown.

Several tree-ring studies have shown stronger increases of $iWUE^{leaf}$ in evergreen needleleaf forests (ENFs) than in deciduous broadleaf forests (DBFs) (Frank et al., 2015; Lavergne et al., 2017; Leonardi et al., 2012). In particular, Frank et al. (2015) detected $iWUE^{leaf}$ increases of $0.22 \pm 0.06\%$ year⁻¹ over the 20th century for 14 ENF sites in European forests, which is consistent with a constant c_i/c_a strategy, but smaller increases of $iWUE^{leaf}$ in the magnitude of $0.14 \pm 0.1\%$ year⁻¹ for nine European DBF sites, more consistent with an intermediate scenario between constant c_a-c_i and constant c_i/c_a . However, the opposite patterns were also found in Saurer et al. (2014), with larger $iWUE^{leaf}$ increases observed in DBFs compared to ENFs in the European region (around $0.32 \pm 0.07\%$ year⁻¹ vs. $0.26 \pm 0.13\%$ year⁻¹, respectively). Nevertheless, the differences between DBF and ENF trends were not significant in either study owing to large site-to-site variability, and the general $iWUE^{leaf}$ trends were still broadly consistent with a constant c_i/c_a . Thus, despite site-to-site variability and/or specific differences in plant responses to changes in c_a , the general patterns of c_i/c_a derived from tree-ring series tend to be more consistent with the strategy of constant c_i/c_a .

Estimates of c_i/c_a at ecosystem scales (denoted c_i^*/c_a) from eddy-covariance studies are not straightforward, but c_i^* can be inferred as first approximation from eddy-covariance-derived ecosystem WUE by inverting Equation (1):

$$c_i^* = c_a - 1.6WUE \quad (10)$$

In contrast to FACE experiments and results from tree-ring studies, the few studies using eddy-covariance flux observations have reported gas exchange strategies more consistent with a constant c_i^* , leading to a decrease in c_i^*/c_a over the past two decades (Keenan et al., 2013; Mastrotheodoros et al., 2017; Wang et al., 2018). Despite strong site-to-site variability and differences in the magnitude of changes, all studies identified a larger decrease in c_i^*/c_a , associated with a larger increase of ecosystem WUE, in DBFs compared to ENFs. In particular, Mastrotheodoros et al. (2017) found increases of $iWUE^{eco}$ of $3.0 \pm 1.9\%$ year⁻¹ in DBFs and $0.3 \pm 0.5\%$ year⁻¹ in ENFs over 1995–2014, while Wang et al. (2018) found increases of $1.93 \pm$

1% year⁻¹ in DBFs and $0.85 \pm 0.6\%$ year⁻¹ in ENFs using the $iWUE^{eco}$ formulation over 1991–2014. The relatively small magnitude of c_i^*/c_a decreases for ENFs suggests that unlike DBFs, ENF trees might follow an intermediate scenario between constant c_i^* and constant c_i^*/c_a with increasing c_a . This would be more consistent with most tree-ring studies.

These various conflicting findings have led some researchers to suggest that rising c_a could result in plants regulating leaf gas exchange along a continuum represented by the three above-mentioned strategies depending on c_a level, life stage, or species. Using isotopic data from both CO₂ enrichment and palaeoecological studies, Voelker et al. (2016) suggested that trees might follow a “dynamic” strategy with c_i/c_a increasing at low c_a (200–400 ppm) and levelling off at higher c_a (400 ppm), helping plants to maximize carbon gain at low c_a while avoiding drought stress at high c_a . This implies that WUE should stay nearly constant or increase slightly at low c_a , but increase proportionally to c_a at higher c_a . Brienen et al. (2017) also suggested that the tree strategy might vary between different developmental stages and species. However, most above-mentioned tree-ring studies investigating $iWUE^{leaf}$ trends over the 20th century have reported leaf gas exchange strategy broadly consistent with a constant c_i/c_a in a period when c_a was lower than 400 ppm, which is in disagreement with the argument proposed by Voelker et al. (2016). Similarly, eddy-covariance studies tend to suggest that c_i^*/c_a has decreased over the past two decades, which is in contradiction to Voelker et al. (2016). Thus, the large differences between WUE trends inferred from the different data sources, especially for DBFs, remain enigmatic. Nevertheless, investigating potential biases in the estimates and quantifying the uncertainties associated with the source data are the first steps towards accurate interpretations of the differences in WUE trends.

3.2 Methodological issues underlying trend estimates

In this section, we investigate potential biases and uncertainties in the estimation of trends related to the methodology applied that might explain part of the discrepancies observed within and across scales. We also compare estimates of WUE trends inferred from tree-ring carbon isotopes and eddy-covariance flux measurements derived from open-access data sets (Table 1) over their common period of records (i.e. 1992–2011).

3.2.1 Stable carbon isotope data

All tree-ring studies have applied Equation (4) for estimating historical trends in $iWUE^{leaf}$. However, the discrimination model represented by Equation (3) is a considerable simplification of the processes involved in determining $\Delta^{13}C$ at the leaf level, and this matters when considering small variations and trends. Plant tissues are constructed from the net pool of carbon captured during metabolism, that is the balance of carbon gain via assimilation and carbon loss via photorespiration and mitochondrial respiration (Wingate et al., 2007). Furthermore, although CO₂ diffuses into the leaf through the stomata,

it has to travel across internal components of the leaf before being fixed in the first step of the Calvin cycle. Photorespiration and day respiration, and the CO₂ transfer from substomatal cavities to the site of fixation (mesophyll conductance), all potentially have an impact on $\Delta^{13}\text{C}$ (Flexas, Ribas-Carbo, Diaz-Espejo, Galmes, & Medrano, 2008). As a result, a substantial fraction of the shift in c_i with increasing c_a reported in several tree-ring studies could be an artefact due to these approaches disregarding these effects on $\Delta^{13}\text{C}$ in Equation (3). Note that the value for b in Equation (3), that is 27‰, was first estimated on leaf bulk material to account for all the isotopic effects related to photosynthetic discrimination and postphotosynthetic fractionation (Cernusak et al., 2009). Nevertheless, this value may vary across different species (see discussions in Schubert & Jahren, 2012; Ubierna & Farquhar, 2014).

The model for $\Delta^{13}\text{C}$ including all these processes is expected to represent $\Delta^{13}\text{C}$ more accurately than Equation (3) when considering the first products of photosynthesis. However, it is substantially more difficult to implement than Equation (3) as it introduces a number of additional terms (Flanagan & Farquhar, 2014; Flexas et al., 2016). It is also difficult to assess values for all the fractionation factors. Several studies have suggested the inclusion at least of the photorespiration term in the model for $\Delta^{13}\text{C}$, as this term contributed to increasing $\Delta^{13}\text{C}$ in agreement with atmospheric and plant observations (Keeling et al., 2017; Schubert & Jahren, 2018). Assuming infinite boundary-layer and mesophyll conductances and negligible fractionation during day respiration as shown by several studies (e.g. Ghashghaie et al., 2003), the model for $\Delta^{13}\text{C}$ can be expressed as:

$$\Delta^{13}\text{C} = a + (b - a) \frac{c_i}{c_a} - f \frac{\Gamma^*}{c_a} \quad (11)$$

where b and f are the fractionations associated with Rubisco carboxylation ($28 \pm 2\text{‰}$) and photorespiration ($12 \pm 4\text{‰}$), respectively (Ubierna & Farquhar, 2014). Γ^* is the CO₂ compensation point in the absence of day respiration, that is the value of c_i at which the rate of photosynthetic CO₂ uptake equals that of photorespiratory CO₂ evolution (Brooks & Farquhar, 1985).

We tested the effect of including the isotopic fractionation due to photorespiration on $\Delta^{13}\text{C}$ (and thus on c_i/c_a) variations using synthetic data (Figure 2a). When c_i/c_a remains constant over a range of c_a , a constant $\Delta^{13}\text{C}$ with c_a increase is expected according to Equation (3), but a $\Delta^{13}\text{C}$ increase should be observed following Equation (11). Rising c_a under a constant $c_a - c_i$ scenario leads to a stronger increase in $\Delta^{13}\text{C}$ using Equation (11) rather than Equation (3). In contrast, the magnitude of changes in $\Delta^{13}\text{C}$ with c_a increase is not significantly different for the two $\Delta^{13}\text{C}$ models in the case c_i remains constant. Neglecting the photorespiration term ($-f\Gamma^*/c_a$) in the discrimination model may thus lead to misinterpretation of how leaf gas exchange strategies vary with changing c_a levels. In the example, the photorespiration

term contributes to increase $\Delta^{13}\text{C}$ with c_a rise by 0.004‰ ppm^{-1} over the range of 285–400 ppm, in a scenario of constant c_i/c_a , which is within the range of variability measured in C_3 plants (Schubert & Jahren, 2018). The trend in $i\text{WUE}^{\text{leaf}}$ tended to be higher using Equation (11) than (3) for a same $\Delta^{13}\text{C}$ (Figure 2b), in agreement with Keeling et al. (2017). Note that here we made the assumptions that only c_i may change with rising c_a , and that T_{air} remains constant over the range of c_a . Thus, the effect on $\Delta^{13}\text{C}$ of changes in Γ^* , through changes in T_{air} , was not considered ($\Gamma^* = 43$ ppm at 25°C ; Bernacchi, Singaas, Pimentel, Portis, & Long, 2001). We acknowledge that potential contributions from other environmental drivers (e.g. plant water availability or nitrogen deposition) may also affect changes in $\Delta^{13}\text{C}$ (see Text S3), but given the large uncertainties in the impact of these effects on $\Delta^{13}\text{C}$, we only considered Equation (11) for assessing the sensitivity of $\Delta^{13}\text{C}$, and thus $i\text{WUE}^{\text{leaf}}$, to changing environmental conditions.

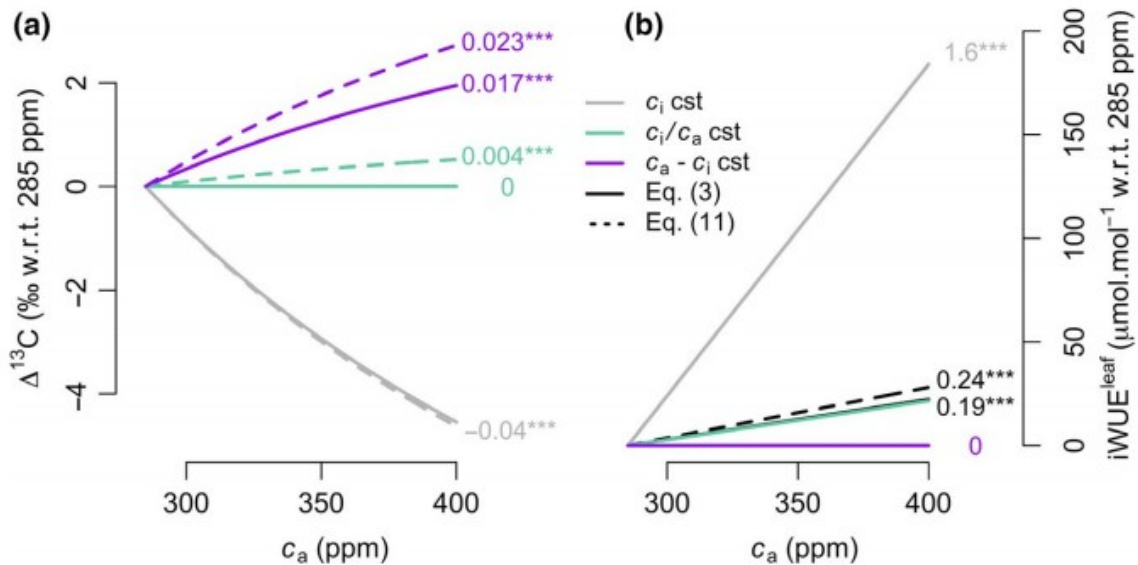


Figure 2. Example of the effect of increasing c_a over the range of 285–400 ppm on $\Delta^{13}\text{C}$ (a) and $i\text{WUE}^{\text{leaf}}$ (b) values relative to respective values at 285 ppm. Calculations are performed on synthetic data for the three different leaf gas exchange “scenarios” using the simple discrimination model (i.e. Equation (3); bold lines) and that considering the photorespiration term only (i.e. Equation (11); dashed lines). The leaf gas exchange scenarios use the following assumptions: (1) $c_i = 199.5$ ppm; (2) $c_i/c_a = 0.7$; and (3) $c_a - c_i = 85.5$ ppm. The values used for the $\Delta^{13}\text{C}$ calculations were $a = 4.4\text{‰}$, $\delta = 27\text{‰}$, $b = 30\text{‰}$, $f = 12\text{‰}$, and $\Gamma^* = 43$ ppm ($T_{\text{air}} = 25^\circ\text{C}$ and $P_{\text{atm}} = 98.716$ kPa). The numbers on the right indicate the respective trend slopes in ‰ increase of $\Delta^{13}\text{C}$ ppm^{-1} increase of c_a (a) and in $\mu\text{mol/mol}$ increase of $i\text{WUE}^{\text{leaf}}$ ppm^{-1} increase of c_a (b). The asterisk denotes the significance of the trend ($p < 0.001$, ***)

Another methodological issue when using stable carbon isotopes in tree rings is related to data preprocessing before assessing $i\text{WUE}^{\text{leaf}}$ trends. Two recent studies (Frank et al., 2015; Xu et al., 2018) performed an empirical adjustment of carbon isotopic series to remove the climate effects on c_i (the so-called τ approach) in an attempt to isolate trends in $i\text{WUE}^{\text{leaf}}$ that are not related to climate change (see Text S4 for more details). The difference between the standard tree-ring c_i and the climate-corrected c_i ($^{cc}c_i$) was used for assessing trends in climate-corrected $i\text{WUE}^{\text{leaf}}$ ($^{cc}i\text{WUE}^{\text{leaf}}$) and for

determining the contributions of rising c_a and climate changes to the $iWUE^{leaf}$ trends. We applied this procedure to a tree-ring network of nine sites available in the ITRDB from temperate forests assessed to be sensitive to evaporative demand (i.e. VPD and T_{air} ; see Table S5) and compared the effect of using either of the two models for $\Delta^{13}C$ (Equations 3 and 11) to infer leaf-level $iWUE$ trends over 1992–2011. We found significantly lower increases in $^{cc}iWUE^{leaf}$ (0.23 ± 0.11 and $0.23 \pm 0.17\%$ year⁻¹, respectively, for Equations 3 and 11) than those estimated without correcting for VPD or T_{air} (0.53 ± 0.36 and $0.49 \pm 0.28\%$ year⁻¹, respectively; median \pm interquartile range; Figure 3). Even though the difference in trends between $iWUE^{leaf}$ estimated from the two $\Delta^{13}C$ models is not significant, the amplitudes of variation in $iWUE^{leaf}$ trends between sites are lower using Equation (11) than Equation (3), suggesting that adding the photorespiration term in the discrimination model tends to reduce the intersite variability and thus the uncertainty. The trend differences between $iWUE^{leaf}$ and $^{cc}iWUE^{leaf}$ suggest that the increasing trend in $iWUE^{leaf}$ is primarily driven by evaporative demand rather than by c_a , consistent with previous studies (Frank et al., 2015; Wang et al., 2018), but differ from other studies which suggest a greater contribution from CO_2 (e.g. Dekker, Groenendijk, Booth, Huntingford, & Cox, 2016). There are several limitations associated with the τ approach, however. First, this procedure assumes that variations in c_i are only driven by c_a and one climate variable and that both drivers have linear and stable effects on c_i over time. Secondly, the sampling strategies for generating the tree-ring series have been chosen so as to maximize the climate signal (thus often favouring old trees from high-latitude and high-elevation forests), and because of this intentional bias, they may not be representative of forests in general. One recent study demonstrated that the tree-ring growth series available in the ITRDB for the US Southwest region tend to overestimate regional forest climate sensitivity by around $50 \pm 9\%$ compared to a spatially unbiased tree-ring network (Klesse et al., 2018). These results suggest that tree-ring-based $iWUE^{leaf}$ trends estimated so far in the literature may only be representative of marginal locations and could overestimate the effect of climate change on regional $iWUE^{leaf}$ trends. Note that without applying the climate correction, the median increase in $iWUE^{leaf}$ calculated here is roughly proportional to the increase of c_a over the same period (Figure 3). It is thus very likely that the τ approach tends to overcorrect tree-ring c_i series, resulting in underestimation of the effect of c_a on the trends. We suggest that this correction should be avoided.

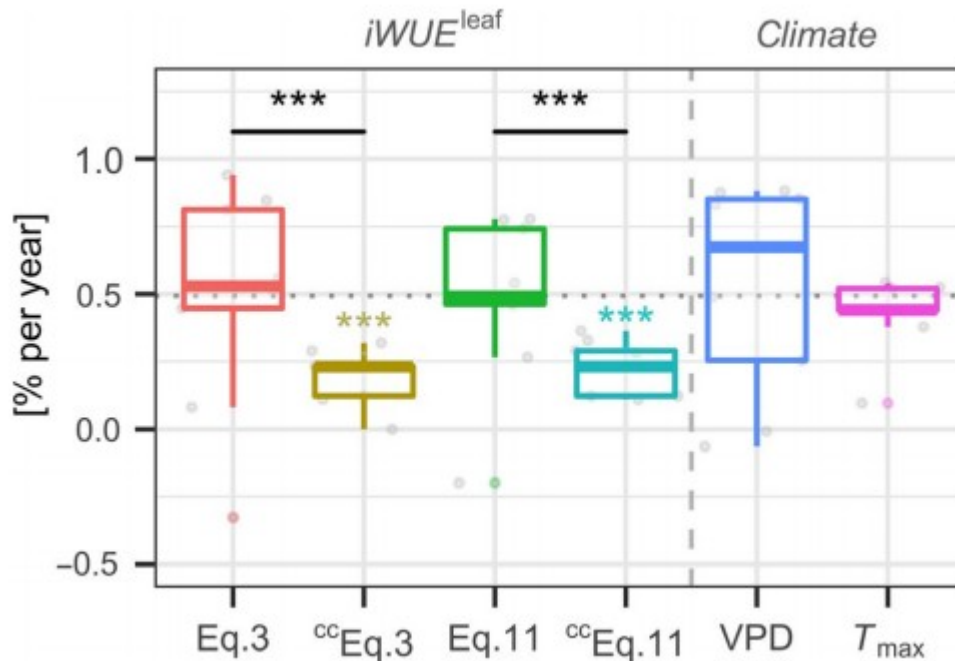


Figure 3. Annual changes in tree-ring-based $iWUE^{\text{leaf}}$ for raw and climate-corrected (cc) series and in climate drivers (i.e. VPD and T_{max}) for the selected nine tree-ring sites available in the ITRDB network with strong influence from evaporative demand (see Table S5). The Sen slopes of the percentage of changes relative to the site mean (% year⁻¹) over 1992–2011 are presented. $iWUE^{\text{leaf}}$ trends inferred from the simple discrimination model (Equation 3) and that including the photorespiration term only (Equation 11) are compared. The values used for calculations were $a = 4.4\text{‰}$, $\bar{h} = 27\text{‰}$, $b = 30\text{‰}$, $f = 12\text{‰}$, and $d = 2.1\text{‰}$. Γ^* was calculated as a function of temperature following Bernacchi et al. (2001). The grey horizontal dotted line is the Sen slope of the percentages of changes in c_a relative to the site mean (% year⁻¹) over 1992–2011 calculated using direct in situ measurements from Scripps (http://scrippsco2.ucsd.edu/data/atmospheric_co2/primary_mlo_co2_record; last update on 12 December 2018). Summer mean T_{max} (°C) and VPD (kPa) were estimated with the CRU TS4.01 gridded data set (Harris, Jones, Osborn, & Lister, 2014). Significant differences between $\Delta WUE/\Delta c_a$ estimates and the 1:1 line (asterisks in colour) and between the different groups of $\Delta WUE/\Delta c_a$ (black lines and asterisks) are also indicated (Student's t test or Wilcoxon test; $p < 0.001$, ***)

Plants preferentially use newly formed photosynthates to form wood, but they can also access decade-old carbon stored in nonstructural carbohydrates (NSCs) when the plant is stressed or physically damaged (Dietze et al., 2014; Hartmann & Trumbore, 2016). The mixing and turnover of old and new NSC might thus dampen the $\delta^{13}\text{C}_{\text{TR}}$ signal used as a proxy of leaf gas exchange (Yi et al., 2018) and should better reflect year-to-decades smoothed rather than interannual changes in $iWUE^{\text{leaf}}$. This is a relatively minor issue when the goal is to estimate long-term trends in $iWUE^{\text{leaf}}$, but given that the effects of NSCs on $\delta^{13}\text{C}_{\text{TR}}$ are still largely unknown (Hartmann & Trumbore, 2016), they increase the degree of uncertainty when estimating $iWUE^{\text{leaf}}$. $\Delta^{13}\text{C}$ can also vary as trees grow in height, giving rise to an effect that has also been called the “juvenile effect” (Francey & Farquhar, 1982; McDowell et al., 2011) but, in reality, one that continues well into maturity. Brienen et al. (2017) demonstrated that this effect is not primarily related to age, as was previously assumed in many studies (Waterhouse et al., 2004;

Young et al., 2012), but rather to height; and that its magnitude depends on the species considered. Increasing tree height might be expected to increase constraints on water transport to the canopy, thereby reducing c_i/c_a and leaf $\Delta^{13}\text{C}$. Developmental effects could potentially confound the interpretation of $i\text{WUE}^{\text{leaf}}$ changes over time inferred from single tree-ring series, as their impacts on $i\text{WUE}^{\text{leaf}}$ can be as large as those from CO_2 and climate (Brienen et al., 2017). These findings have major implications when using tree-ring series to infer $i\text{WUE}^{\text{leaf}}$, as they indicate that $\Delta^{13}\text{C}$ should be corrected for developmental effects over the tree's lifespan. It is, however, unknown where (at what height) in a deep crown the photosynthates measured in a ground-level tree-ring series are produced, complicating the implementation of this correction.

3.2.2 Eddy-covariance data

In general, the mean ecosystem WUE trends inferred from the eddy-covariance studies are based on a rather small proportion of the total network (20–26 eddy-covariance sites; Keenan et al., 2013; Mastrotheodoros et al., 2017; Wang et al., 2018). Of the 212 sites available in the FLUXNET-2015 (Tier 1) network, only 39 stations representing DBF, ENF, evergreen broadleaved (EBF), or mixed (MF) forests have seven or more years of available flux measurements (i.e. less than a fifth of the total network). Of these 39 stations, 36 are located in North America and Europe, so changes in ecosystem WUE in other regions are not open to investigation using available data. It will be scientifically important to increase the number of eddy-covariance stations in remote regions; to continue support for stations in those regions that have long data records; and to make existing data records available from flux towers in other parts of the world.

In contrast to tree-ring studies, which have used a common definition for WUE, eddy-covariance studies have applied several different formulations for ecosystem WUE—resulting in different reported WUE trends from the same sites (Figure 1). Here, we calculated changes in WUE^{eco} , $i\text{WUE}^{\text{eco}}$, and IWUE^{eco} over 1992–2011 for 25 eddy-covariance stations from the FLUXNET-2015 (Tier 1) product representing ENFs (15 stations) and DBFs (10 stations), after further data screening, quality checking, and data processing (see Text S2 and Table S6). Although the differences between trend estimates are not significant, the strongest increasing trend is found for IWUE^{eco} , followed by $i\text{WUE}^{\text{eco}}$ and WUE^{eco} (1.44 ± 3.94 , 1.17 ± 5.16 , and $0.88 \pm 2.70\% \text{ year}^{-1}$, respectively; median \pm interquartile range; Figure 4a), broadly consistent with estimates from recent studies (Mastrotheodoros et al., 2017; Wang et al., 2018).

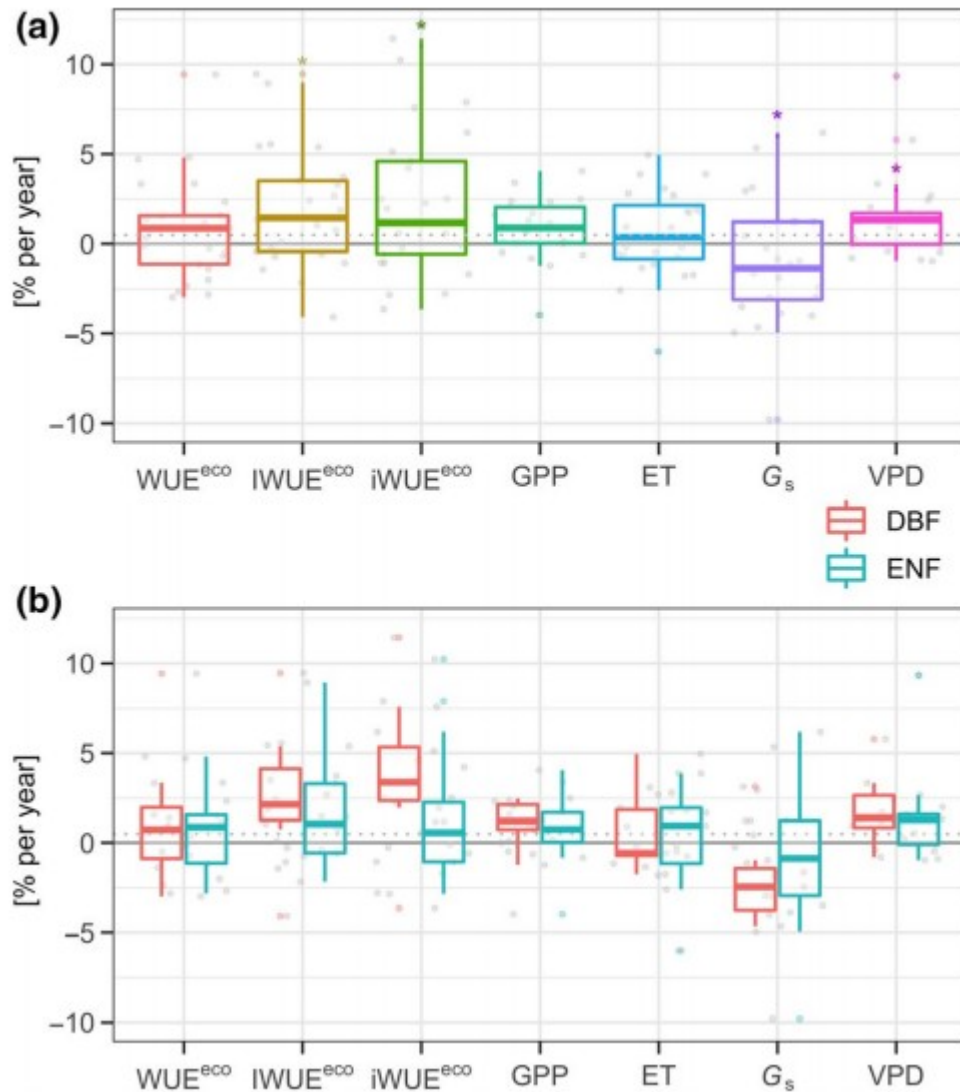


Figure 4. Annual changes in WUE^{eco} , $IWUE^{eco}$, $iWUE^{eco}$, GPP, ET, G_s , and VPD for DBF and ENF forests over 1992–2011 from a network of 25 eddy-covariance stations derived from FLUXNET-2015 (Tier 1) product (see Table S6). The Sen slopes of the percentages of changes in the variables relative to the site mean (% year⁻¹) over 1992–2011 are presented. The grey horizontal dotted line is the Sen slope of the percentages of changes in c_a relative to the site mean (% year⁻¹) over 1992–2011 calculated using direct in situ measurements from Scripps (http://scrippsco2.ucsd.edu/data/atmospheric_co2/primary_mlo_co2_record; last update on 12 December 2018). In (a) are the general estimates, and in (b) are the estimates for the two PFTs considered. All the calculations were performed using the “Bigleaf” R package (see Text S2; Knauer, El-Madany, et al., 2018). Significant differences between estimates and the 1:1 line (asterisks) are also indicated (Student *t* test or Wilcoxon test; $p < 0.05$, *)

Data processing choices could potentially affect the estimates of ecosystem WUE trends, and thus must be made carefully with a full assessment of the uncertainty involved. Such choices include the flux partitioning method selected to infer GPP (daytime vs. night-time methods), the assumptions made when isolating the transpiration flux (exclusion of rainy days and the following 24 or 48 hr), the definition for the growing season (predefined

summer months vs. GPP filter), and the procedure to aggregate observations in time (average, median, or sum). Using the median ecosystem WUE value per year and site is recommended because this metric is less sensitive to very small or large values for the variables at the half-hourly or hourly scale, and less sensitive to outliers (Mastrotheodoros et al., 2017). Also, given the large variations in ecosystem WUE trends between sites, the selection of stations to include in the estimates may also influence the final result. Assuming that longer records are more reliable in the slope estimations, weighting the slopes by the time-series length may provide more accurate estimates of WUE trends. In the example here, this would lead to slightly lower increases of ecosystem WUE with lower intersite variability (1.36 ± 3.73 , 1.17 ± 4.79 , and $0.36 \pm 2.66\%$ year⁻¹, respectively, for $iWUE^{eco}$, $iWUE^{eco}$, and WUE^{eco}).

Consistent with previous studies, Figure 4b suggests that DBFs tend to show larger increases in ecosystem WUE than ENFs, mainly due to the larger decrease of G_s or ET and increase of VPD in DBFs compared to ENFs. Note, however, that the differences in ecosystem WUE trends between PFTs are not significant. Nevertheless, the strong increasing trends in ecosystem WUE appear to be mainly driven by DBFs. We acknowledge that the relatively short length of the current eddy-covariance records is a major limitation for long-term studies; nevertheless, this demonstrates that the trend estimates are likely not representative of processes occurring at the global scale (Knauer et al., 2017) and caution should be used when interpreting trends using eddy-covariance flux measurements for a given selection of sites.

3.2.3 Impacts of the different sources of uncertainty on the trend estimates

From the foregoing discussion, it should be clear that methodological approaches and assumptions have significant impacts on estimated WUE trends. Systematic quantification of uncertainties is required. Here, $iWUE^{leaf}$ inferred from carbon isotopes in tree rings over 1992–2011 increased by around $0.5 \pm 0.3\%$ year⁻¹, while $iWUE^{eco}$ inferred from eddy-covariance measurements increased by around $1.2 \pm 5\%$ year⁻¹ over the same period. Because the range of intersite variability of the tree-ring-based estimates falls within that of eddy-covariance records, no conclusion can be drawn on whether the differences between the two types of data sets are universal. These findings have, however, some limitations because the sites considered for the calculations were different for the tree-ring or eddy-covariance data. Nevertheless, this is an important result as it suggests that the trend estimates have large uncertainties simply based on the large variations among sites.

Other sources of uncertainty may affect estimates of WUE, including random and systematic errors for the eddy-covariance flux measurements (Knauer, Zaehle, et al., 2018; Richardson et al., 2012), and analytical error and sampling bias for stable carbon isotopes in tree rings (Daux et al., 2018; Gessler et al., 2014). However, these sources of uncertainty do not

necessarily have an impact on trends (see Table 2). Random and analytical errors should not affect trends, unless their characteristics change over time. Biases due to flux partitioning methods applied are typically site-specific and expected to be temporally stationary. A site mean GPP could be artificially low or high (Wehr et al., 2016), but there is no evidence for a temporal trend in partitioning bias. Knauer, Zaehle, et al. (2018) showed that systematic errors related to energy balance nonclosure or assumption of a full aerodynamic coupling between the vegetation and the atmosphere (i.e. infinite G_a) may be more critical for the accurate estimation of ecosystem WUE than those related to the selection of flux partitioning algorithm or the contribution from nontranspirational water fluxes (i.e. E_v or I_n) to ET. We investigated whether the magnitude of the energy balance nonclosure (i.e. residual = $R_n - (LE + H)$) changed over time at each eddy-covariance site. Despite year-to-year variability in the residuals, the trends in the residuals were not significant for most sites (not shown), suggesting that energy balance nonclosure probably does not significantly affect estimated WUE trends. Nevertheless, the different magnitudes of change in WUE^{eco} and $iWUE^{eco}$ found here suggest that assumptions about the aerodynamic conductance may be important. Note that many uncertainties affect both LE and GPP, but because the two are coupled (when excluding nontranspirational fluxes as far as possible), the variability in GPP/LE is much lower than that of either. Thus, year-to-year variability in ecosystem WUE is relatively low compared to that in GPP and LE, giving a higher signal-to-noise ratio.

TABLE 2 Sources of uncertainty in the estimates of WUE associated with the different data sets, and their impacts on estimated trends

Type of data	Source of uncertainty	Examples and estimation of errors	Impacts on trends
Stable carbon isotopes in tree rings	Analytical error	Instrumental drift and low measurement reproducibility: rejection of outliers using an internal laboratory standard (typically $\pm 0.1\text{‰}$)	No impact as considered constant over time
		Sampling bias	Large intrasite variability and low number of sampled trees: consideration of series with high expressed population signal (>0.85) and low confidence interval around the mean ($<0.5\text{‰}$)
	Conceptual uncertainty	Selection of sites at high latitude and high elevation: difficult to quantify	Medium as trend estimates may only reflect changes at marginal locations
		Assumptions of infinite boundary-layer and mesophyll conductances and negligible fractionation during day respiration in the $\Delta^{13}\text{C}$ model: difficult to quantify	Low to medium
		Values for fractionation factors in the $\Delta^{13}\text{C}$ model	No impact as assumed constant over time
		Mixing and turnover of old and new nonstructural carbohydrates: difficult to quantify	Low as tree-ring proxy of year-to-decades changes in $i\text{WUE}^{\text{leaf}}$
Eddy-covariance flux measurements	Random error	Site-specific: estimated in the FLUXNET data	No impact as assumed constant over time
		Systematic error	Energy balance nonclosure ($\text{LE} + \text{H} < R_n$) propagated to G_s and thus to $i\text{WUE}^{\text{eco}}$: analysis of temporal changes in the residuals
	Conceptual uncertainty	NEE partitioning propagated into GPP: systematic over- or underestimation of GPP	No impact or low as assumed constant over time
		Assumptions of infinite G_s and vegetation-atmosphere coupling for WUE^{eco} and $i\text{WUE}^{\text{eco}}$ estimates: comparison of the two estimates	Medium
		Assumptions of negligible S and G for $i\text{WUE}^{\text{eco}}$: overestimation of $i\text{WUE}^{\text{eco}}$	No impact or low as assumed constant over time

4 WUE TRENDS PREDICTED BY VEGETATION MODELS AND AGREEMENTS WITH OBSERVATIONS

Several attempts have been made to simulate WUE trends over the past century with different vegetation models and to compare the resulting trends with observations from tree rings and eddy-covariance flux towers (Figure 1; Tables S1–S4). Saurer et al. (2014) indicated a weaker magnitude of $i\text{WUE}^{\text{leaf}}$ increase in simulations from the Land Surface Processes and Exchanges version 1.0 (LPX-Bern1.0) model than in the tree-ring-inferred trends. Similarly, different model simulations from the Coupled Model Intercomparison Project phase 5 (CMIP5) reported an increase of WUE^{eco} of $14 \pm 12\%$ between 1860 and 2005, a large underestimate compared to the increase of around 40% inferred from a combination of tree-ring and eddy-covariance data over the same period (Dekker et al., 2016). Frank et al. (2015) did not find statistically different $i\text{WUE}^{\text{leaf}}$ values and trends between DBF and ENF in simulations by several vegetation models, although slight differences were observed in their tree-ring-based estimates. Keller et al. (2017) showed that while the LPX-Bern1.3 model, an updated version of the model used by Saurer et al. (2014), simulated an increase of $i\text{WUE}^{\text{leaf}}$ of

around 25% for the 20th century (similar to that observed in a global tree-ring data set), the Community Land Model version 4.5 (CLM4.5) (Raczka et al., 2016) simulated much stronger increases in $iWUE^{leaf}$ than are compatible with the tree-ring data. Liu et al. (2015) found good agreement between simulations of WUE^{eco} from the Boreal Ecosystem Productivity Simulator (BEPS) model and eddy-covariance measurements, but the model underestimated the highest WUE^{eco} values. Thus, it appears that vegetation models disagree with one another in their simulations of WUE changes, but share a tendency to underestimate observed WUE trends (Figure 1b).

Differences among simulations of WUE trends are very likely due to the different structures of the models (see also Text S5). Vegetation models make a variety of different assumptions about the processes influencing WUE, including the fundamental leaf-level gas exchange responses to varying c_a , the degree of coupling to the leaf/atmospheric boundary layer, the treatment of canopy water interception, and the impact of soil moisture stress on A and g_s (see review from De Kauwe et al., 2013). As a result, the sensitivity of models to rising c_a differs greatly between models, although all of them predict an increase in WUE that is less than proportional to the c_a increase (Figure 1b). Vegetation models also incorporate different water stress functions (Medlyn et al., 2016) and so interpreting simulated WUE trends may depend on the relative stress levels simulated across models. However, many parameter values for these “mechanistic” models are not easily determined, increasing the uncertainties in the estimates.

Given the mismatch between observations and simulations and the discrepancies between different data sources and different vegetation models, new approaches are evidently needed. CMIP phase 6 (CMIP6) has strongly recommended the implementation of carbon isotope formulations in models (Jones et al., 2016). Carbon isotopes have been included in a few models, including LPJ (Scholze, Ciais, & Heimann, 2008), LPX-Bern (Keller et al., 2017; Saurer et al., 2014), and CLM4.5 (Duarte et al., 2017; Keller et al., 2017; Raczka et al., 2016). In general, however, vegetation models calculate $\Delta^{13}C$ following the simple model of Farquhar, O'Leary, et al. (1982), that is Equation (3), and the simulations are then compared to $\Delta^{13}C$ directly inferred from tree rings using Equation (2). It should be clear from the foregoing discussion that neither of these simplifications is adequate for the analysis of recent trends. In a recent study by Keller et al. (2017), LPX-Bern1.3 simulated 20th-century trends in $iWUE^{leaf}$ in reasonable agreement with a global tree-ring data set, but the decrease in $\Delta^{13}C$ was underestimated. Similarly, incorrect simulations of $\Delta^{13}C$ were produced by CLM4.5, likely resulting from both the g_s parameterization and the nitrogen limitation scheme (Keller et al., 2017; Raczka et al., 2016). By adjusting model parameters controlling leaf area index, g_s , and soil water availability within CLM4.5, Duarte et al. (2017) simulated $\Delta^{13}C$ in better agreement with site observations, demonstrating that stable carbon isotope data could be used in combination with eddy-covariance flux measurements for evaluating and

improving models. However, some of these studies performed ad hoc calibrations in order to improve agreement between simulations and observations. Raczka et al. (2016) used an empirical photosynthesis scaling to provide a better match of simulated GPP and R_{eco} to eddy-covariance flux measurements. Duarte et al. (2017) manually adjusted the model parameters to better fit simulations and observations of GPP and ET. Such strategies are not recommended as they invite the possibility of error compensations if important processes are missing or incorrectly represented. They do not ensure that the model is reliable. Major improvements in the process-level evaluation of models are still needed in order to increase their predictive skill.

5 CONCLUSIONS AND PERSPECTIVES

Continuing environmental changes are affecting the stomatal regulation of leaf gas exchange, but the extent to which the resulting physiological changes translate into changes in leaf- to ecosystem-level WUE remains unclear. Assessing the magnitude of WUE changes over time is challenging because of (a) uncertainties related to the data sources used to infer trends, (b) unresolved differences between results obtained from the different data sources, and (c) different modelling approaches and assumptions for WUE.

Based on this review, several recommendations can be made for improving long-term observation-based estimates of WUE and thus better informing vegetation models: (a) given the large and often underestimated uncertainties related to the different data sources, we propose systematically incorporating them in the assessment of “observed” trends; (b) given that estimating leaf-level iWUE using Equation (11) reduces the intersite variability (Figure 3), we suggest to consider at least the photorespiratory term in the discrimination model for assessing trends; (c) given the different assumptions underlying the estimations of G_a and G_s that increase uncertainties in ecosystem iWUE, we recommend systematically comparing the different formulations, that is Equations (5), (6), and (8), to better interpret temporal trends in WUE; and (d) most importantly, stronger scientific exchanges and collaborations between the tree-ring and eddy-covariance flux communities are recommended. Adding more dendroisotope studies at eddy-covariance sites would certainly help in understanding the physiological mechanisms underlying ecosystem fluxes, which eddy-covariance data alone cannot address.

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