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

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**Summary**

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 dataset 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 and improve the representation of WUE in vegetation models.

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

**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 (*Tr*) (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 et al., 2016). These indirect effects include changes in leaf area index and canopy structure (Gerten et al., 2008) and atmospheric boundary layer feedbacks (De Kauwe et al., 2013; Field, Jackson, & Mooney, 1995). Thus, scaling up the terrestrial carbon and water response to environmental stimuli from leaf to canopy and ecosystem is a key uncertainty (De Kauwe et al. 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 multi-centennial 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 et al., 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 et al., 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 datasets 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 dataset 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.

1. **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 et al., 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, carbon and water fluxes (Hobeichi et al., 2018; Parazoo et al., 2014), because none of the datasets 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 et al., 2014), but here we focus on the most commonly used to assess long-term trends.

* 1. **Leaf level estimates**

At the leaf level, the ‘intrinsic’ WUE (iWUEleaf, μmol mol–1) is defined as the ratio of photosynthetic assimilation rate (*A*, μmol m-2 s-1) to stomatal conductance for H2O diffusion (*g*sw, mol m-2 s–1)as follows(Ehleringer, Hall, & Farquhar, 1993):

(1)

where *g*sc is the stomatal conductance for CO2 diffusion (mol m-2 s­–1) and, *c*i and *c*a are the leaf-intercellular and ambient CO2 concentrations, respectively (μmol 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 iWUEleaf is implicit, as it controls the ratio of *c*i to *c*a. Equation (1) predicts that iWUEleaf 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 (∆13C). The calculation of ∆13C (‰) requires only knowledge of δ13C of the plant material (δ13CP, 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 (δ13Catm):

(2)

In C3 plants, ∆13C depends principally on the gradient of concentration of CO2 from the external atmosphere to the intercellular spaces, and therefore on *c*i/*c*a. Thus, estimating *c*i/*c*a from δ13C measured in tree rings can provide evidence for changes in iWUEleaf (Table 1).

By far the most commonly used formula to estimate the *c*i/*c*a ratio from ∆13C is the following simple expression (Farquhar, O’Leary, & Berry, 1982):

(3)

where *a* represents the fractionation due to CO2 diffusion in air (4.4‰: Craig, 1953) and is the apparent net fractionation by Rubisco during carboxylation (27‰: Farquhar, Ball, et al., 1982). Equation (3) is convenient because it implies that any change in ∆13C 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 post-photosynthetic 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 post-photosynthetic fractionation processes and quantify uncertainties associated with the estimation of iWUEleaf using tree-rings. Equations (1) and (3) may thus be combined to give iWUEleaf in terms of ∆13C adjusted for post-photosynthetic fractionation processes as in Frank et al. (2015):

(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 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, iWUEleaf 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 et al., 2008). Nevertheless, even though iWUEleaf excludes the direct influence of *D*, it is still dependent on *D,* through changes in *g*s (Cowan & Farquhar, 1977). So comparisons of iWUEleaf should ideally be restricted to situations with similar *D* (Franks et al., 2013).

* 1. **Ecosystem scale estimates**

At the ecosystem scale, the simplest definition of ecosystem WUE is WUEeco (gC kg H2O–1), defined as (Law et al., 2002):

(5)

where GPP is gross primary production (gC m–2 s–1) and ET is evapotranspiration   
(kg H2Om–2 s–1), which includes contributions from rainfall interception (*In*) (water that is evaporated from the canopy surface), bare-ground evaporation (*Ev*), and *Tr*. 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 iWUEeco, defined as:

(6)

where *G*s is the bulk surface conductance of the ecosystem (m s­–1 or mol m–2 s–1) calculated by inverting the Penman-Monteith equation (Monteith, 1965):

(7)

where *G*a is aerodynamic conductance (m s–1), *Rn* 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–1), *ρ* is the density of air (kg m–3), *cp* is the specific heat of air   
(J kg–1 K–1), and *γ* is the psychrometric constant (Pa K–1).

As *G*s can only be estimated indirectly, the alternative ‘inherent’ ecosystem WUE, or IWUEeco, 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):

(8)

where *Patm* 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 *Tr* in all but the sparsest canopies (*Tr* >> *In* + *Ev* when 1 to 2 days after rains are ignored to minimize the other contributions to the total ET flux), WUEeco, IWUEeco and iWUEeco are all expected *a priori* to change in response to environmental variations in a similar fashion to iWUEleaf, when not considering interactions or feedbacks.

Most variables needed to infer ecosystem WUE from equations (5) to (8) can be estimated from eddy-covariance flux measurements above vegetation canopies. Flux towers measure the net ecosystem exchange of CO2 (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 *Reco* from measured NEE (Lasslop et al., 2010; Reichstein et al., 2012; see Text S2 for further details). ET is derived from LE by:

(9)

where λv is the latent heat of vaporization, i.e. the amount of energy required to evaporate 1 kg liquid water (kJ kg–1), 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 non-stomatal 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*aand *G*s from the eddy-covariance flux observations (Knauer, El-Madany, et al., 2018) – but all methods to date necessitate a ‘big-leaf’ assumption in the inversion of *G*s, which introduces additional assumptions.

1. **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 et al., 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 iWUEleaf of 0.2 ± 0.1% yr–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 (Fig. 1b). Some of these studies have shown a weakening iWUEleaf response to *c*a towards the end of the 20th century in regions where both increases in temperature and in *D* may have offset the increase in iWUEleaf 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% yr–1 over the period 1995- 2010 (Keenan et al., 2013), albeit with a slight decrease in magnitude over recent years (i.e. 1.3 ± 0.1% yr–1 over 1991-2014; Mastrotheodoros et al., 2017; Wang et al., 2018). These studies reported a much stronger increase in ecosystem WUE relative to *c*a rise than that shown by tree-ring studies (Fig. 1b). Note that, however, changes in ecosystem-level WUE based on eddy-covariance data were estimated using different formulations, i.e. 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 (Fig. 1b).

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.

* 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 et al., 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 CO2 concentrations across the stomata to maintain a constant *c*i/*c*a (Masle, Farquhar, & Gifford, 1990; Polley et al., 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, & 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; (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 iWUEleaf with decreasing *c*i/*c*a, augmenting the effect of increasing *c*a; the second scenario (ii) implies constant iWUEleaf with increasing *c*i/*c*a,counteracting the effect of increasing *c*a; the third scenario (iii) is intermediate, with iWUEleaf 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, i.e. constant *c*i/*c*a (Ainsworth & Long, 2005; De Kauwe et al., 2013; Gimeno et al., 2016), 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 iWUEleaf, or between the constant *c*a – *c*i and constant *c*i/*c*a scenarios, associated with relatively moderate increase in iWUEleaf (Andreu-Hayles et al., 2011; Lavergne et al., 2017; Leonardi et al., 2012; Urrutia-Jalabert et al., 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 iWUEleaf in species with more rigid leaf architecture, including most gymnosperms (Niinemets et al., 2009, 2011). The combined impacts of these phylogenetic differences on changes in *c*i/*c*a and iWUEleaf are largely unknown.

Several tree-ring studies have shown stronger increases of iWUEleaf in evergreen needleleaf forests (ENF) than in deciduous broadleaf forests (DBF) (Frank et al., 2015; Lavergne et al., 2017; Leonardi et al., 2012). In particular, Frank et al. (2015) detected iWUEleaf increases of 0.22 ± 0.06% yr-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 iWUEleaf in the magnitude of 0.14 ± 0.1% yr-1 for 9 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 iWUEleaf increases observed in DBFs compared to ENFs in the European region (around 0.32 ± 0.07% yr-1 versus 0.26 ± 0.13% yr-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 iWUEleaf trends were still broadly consistent with a constant *c*i/*c*a. Thus, despite site-to-site variability and/or specific differences in plants 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):

(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 IWUEeco of 3.0 ±1.9% yr-1 in DBFs and 0.3 ± 0.5% yr-1 in ENFs over 1995-2014, while Wang et al. (2018) found increases of 1.93 ± 1% yr-1 in DBFs and 0.85 ± 0.6% yr-1 in ENFs using the iWUEeco 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*aincreasing at low *c*a (200 to 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 iWUEleaf 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*ahas decreased over the past two decades, which is in contradiction with 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 these differences in WUE trends.

* 1. **Methodological issues underlying trends 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 datasets (Table 1) over their common period of records (i.e. 1992-2011).

* + 1. **Stable carbon isotope data**

All tree-ring studies have applied equation (4) for estimating historical trends in iWUEleaf. However, the discrimination model represented by equation (3) is a considerable simplification of the processes involved in determining ∆13C 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, i.e., 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 ∆13C (Flexas et al., 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 Δ13C in equation (3). Note that the values for in equation (3), i.e. 27‰, was first estimated on leaf bulk material to account for all the isotopic effects related to photosynthetic discrimination and post-photosynthetic fractionation (Cernusak et al., 2009). Nevertheless, this value may varies across different species (see discussions in Schubert & Jahren, 2012; Ubierna & Farquhar, 2014).

The model for ∆13C including all these processes is expected to represent ∆13C 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 Δ13C, as this term contributed to increasing Δ13C 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 ∆13C can be expressed as:

(11)

where *b* and *f* are the fractionations associated with Rubisco carboxylation (28 ± 2‰) and photorespiration (12 ± 4‰), respectively (Ubierna & Farquhar, 2014). Γ\* is the CO2 compensation point in the absence of day respiration, i.e. 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 Δ13C (and thus on *c*i/*c*a) variations using synthetic data (Fig. 2a). When *c*i/*c*a remains constant over a range of *c*a, a constant Δ13C with *c*a increase is expected according to equation (3), but a Δ13C increase should be observed following equation (11). Rising *c*a under a constant *c*a – *c*i scenario leads to a stronger increase in Δ13C using equation (11) rather than equation (3). In contrast the magnitude of changes in Δ13C with *c*a increase is not significantly different for the two Δ13C models in the case *c*i remains constant. Neglecting the photorespiration term (-*f*Γ\*/*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 Δ13C with *c*a rise by 0.004‰ ppm-1 over the range of 285 to 400 ppm, in a scenario of constant *c*i/*c*a, which is within the range of variability measured in C3 plants (Schubert & Jahren, 2018). The trend in iWUEleaf tended to be higher using equation (11) than equation (3) for a same Δ13C (Fig. 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 *Tair* remains constant over the range of *c*a. Thus, the effect on Δ13C of changes in Γ\*, through changes in *T*air, were not considered (Γ\* = 43 ppm at 25°C; Bernacchi et al., 2001). We acknowledge that potential contributions from other environmental drivers (e.g. plant water availability or nitrogen deposition) may also affect changes in Δ13C (see Text S3), but given the large uncertainties in the impact of these effects on Δ13C, we only considered equation (11) for assessing the sensitivity of Δ13C, and thus iWUEleaf, to changing environmental conditions.

Another methodological issue when using stable carbon isotopes in tree-rings is related to data pre-processing before assessing iWUEleaf 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 iWUEleaf 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 iWUEleaf (cciWUEleaf) and for determining the contributions of rising *c*a and climate changes to the iWUEleaf trends. We applied this procedure to a tree-ring network of 9 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 Δ13C (equations 3 and 11) to infer leaf-level iWUE trends over 1992-2011. We found significant lower increases in cciWUEleaf (0.23 ± 0.11 and 0.23 ± 0.17% yr-1, respectively for equation 3 and 11) than those estimated without correcting for VPD or *T*air (0.53 ± 0.36 and 0.49 ± 0.28% yr-1, respectively; median ± interquartile range, Fig. 3). Even though the difference in trends between iWUEleaf estimated from the two Δ13C models are not significant, the amplitudes of variation in iWUEleaf 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 inter-site variability and thus the uncertainty. The trend differences between iWUEleaf and cciWUEleaf suggest that the increasing trend in iWUEleaf is primarily driven by evaporative demand rather than by *c*a, consistent with previous studies (Frank et al., 2015; Wang et al., 2018), but differs from other studies suggesting a greater contribution from CO2 (e.g. Dekker et al., 2016). There are several limitations associated to 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 ± 9% compared to a spatially unbiased tree-ring network (Klesse et al., 2018). These results suggest that tree-ring-based iWUEleaf trends estimated so far in the literature may only be representative of marginal locations, and could overestimate the effect of climate change on regional iWUEleaf trends. Note that without applying the climate correction, the median increase in iWUEleaf calculated here is roughly proportional to the increase of *c*a over the same period (Fig. 3). It is thus very likely that the τ approach tend 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.

Plants preferentially use newly-formed photosynthates to form wood, but they can also access decade-old carbon stored in non-structural 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 δ13CTR 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 iWUEleaf. This is a relatively minor issue when the goal is to estimate long-term trends in iWUEleaf, but given that the effects of NSCs on δ13CTR are still largely unknown (Hartmann & Trumbore, 2016), they increase the degree of uncertainty when estimating iWUEleaf. ∆13C 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 ∆13C. Developmental effects could potentially confound the interpretation of iWUEleaf changes over time inferred from single tree-ring series, as their impacts on iWUEleaf can be as large as those from CO2 and climate (Brienen et al., 2017). These findings have major implications when using tree-ring series to infer iWUEleaf, as they indicate that ∆13C 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.

* + 1. **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; 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 (Fig. 1). Here, we calculated changes in WUEeco, iWUEeco and IWUEeco 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 trends estimates are not significant, the strongest increasing trend is found for IWUEeco, followed by iWUEeco and WUEeco (1.44 ± 3.94, 1.17 ± 5.16 and 0.88 ± 2.70 % yr-1, respectively; median ± interquartile range, Fig. 4a), broadly consistent with estimates from recent studies (Mastrotheodoros et al., 2017; Wang et al., 2018).

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 versus nighttime methods), the assumptions made when isolating the transpiration flux (exclusion of rainy days and the following 24 or 48 hours), the definition for the growing season (pre-defined summer months versus 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 inter-site variability (1.36 ± 3.73, 1.17 ± 4.79 and 0.36 ± 2.66 % yr-1, respectively for IWUEeco, iWUEeco and WUEeco).

Consistent with previous studies, Fig. 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.

* + 1. **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, iWUEleaf inferred from carbon isotopes in tree-rings over 1992-2011 increased by around 0.5 ± 0.3% yr-1, while iWUEeco inferred from eddy-covariance measurements increased by around 1.2 ± 5% yr-1 over the same period. Because the range of inter-site variability of the tree ring-based estimates fall within that of eddy-covariance records, no conclusion can be drawn on whether the differences between the two types of datasets 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 non-closure 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 non-transpirational water fluxes (i.e. *E*v or *I*n) to ET. We investigated whether the magnitude of the energy balance non-closure (i.e. residual = Rn – (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 non-closure probably does not significantly affect estimated WUE trends. Nevertheless, the different magnitudes of change in IWUEeco and iWUEeco 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 non-transpirational 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.

1. **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 (Fig. 1; Tables S1-S4). Saurer et al. (2014) indicated a weaker magnitude of iWUEleaf 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 WUEeco of 14 ± 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 iWUEleaf 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 iWUEleaf of around 25% for the 20th century (similar to that observed in a global tree-ring dataset), the Community Land Model version 4.5 (CLM4.5) model (Raczka et al., 2016) simulated much stronger increases in iWUEleaf than are compatible with the tree-ring data. Liu et al. (2015) found good agreement between simulations of WUEeco from the Boreal Ecosystem Productivity Simulator (BEPS) model and eddy-covariance measurements, but the model underestimated the highest WUEeco 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 (Fig. 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 (Fig. 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 ∆13C following the simple model of Farquhar, O’Leary, et al., (1982), i.e. equation (3), and the simulations are then compared to ∆13C 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 iWUEleaf in reasonable agreement with a global tree-ring dataset, but the decrease in ∆13C was underestimated. Similarly, incorrect simulations of ∆13C 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 ∆13C 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 observations. 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.

1. **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: (i) Given the large and often underestimated uncertainties related to the different data sources, we propose systematically incorporating them in the assessment of “observed” trends. (ii) Given that estimating leaf-level iWUE using equation (11) reduces the inter-site variability (Fig. 3), we suggest to consider at least the photorespiratory term in the discrimination model for assessing trends. (iii) 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, i.e. equations (5), (6) and (8), to better interpret temporal trends in WUE. (iv) 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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**Tables**

**Table 1.** Commonly used formulations of WUE, and measurements used for inferring trends

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Form of WUE | Spatial scale | Short name | Equation | Temporal scale | Measurements used1 | Data processing | Open-access products2 |
| ‘intrinsic’ | Leaf | iWUEleaf | 4 | Seasonal to yearly | Stable carbon isotopic composition of bulk wood or α cellulose in tree rings (δ13CTR) + atmospheric δ13CO2 | Calculation of Δ13C; corrections for post-photosynthetic fractionations (*d*) and inter-site variability (ε) | ITRDB + Graven et al. (2017) |
| Ecosystem | iWUEeco | 6 | Half-hourly or hourly | Eddy-covariance fluxes (LE, *H*, NEE, GPP) + *R*n, *T*air, VPD, *c*a | Flux partitioning and  data post processing  (see Text S2) | FLUXNET-2015 Tier 1 |
| ‘instantaneous’ | Ecosystem | WUEeco | 5 |
| ‘inherent’ | Ecosystem | IWUEeco | 8 |

1Latent 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). 2International tree-ring databank (ITRDB; <https://www.ncdc.noaa.gov/dataaccess/paleoclimatology-data/datasets/tree-ring>), eddy-covariance flux towers network (FLUXNET; <http://fluxnet.fluxdata.org/data>)

**Table 2.** Sources of uncertainty in the estimates of WUE associated with the different datasets, 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 ± 0.1‰) | No impact as considered constant over time |
| Sampling bias | Large intra-site 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‰) | No impact assuming that the confidence interval around the mean constant over time |
| Selection of sites at high-latitude and high-elevation: difficult to quantify | Medium as trend estimates may only reflect changes at marginal locations |
| Conceptual uncertainty | Assumptions of infinite boundary-layer and mesophyll conductances and negligible fractionation during day respiration in the Δ13C model: difficult to quantify | Low to medium |
| Values for fractionation factors in the Δ13C model | No impact as assumed constant over time |
| Mixing and turnover of old and new non-structural carbohydrates: difficult to quantify | Low as tree-ring proxy of year-to-decades changes in iWUEleaf |
| Magnitude of height effect: difficult to quantify | No impact to medium depending on species |
| Eddy-covariance flux measurements | Random error | Site-specific: estimated in the FLUXNET data | No impact as assumed constant over time |
| Systematic error | Energy non-closure balance (LE + H < Rn) propagated to *G*s and thus to iWUEeco: analysis of temporal changes in the residuals | No impact or low as no significant change of energy non-closure balance over time |
| NEE partitioning propagated into GPP: systematic over- or under-estimation of GPP | No impact or low as assumed constant over time |
| Conceptual uncertainty | Assumptions of infinite *G*a and vegetation-atmosphere couplingfor WUEeco and IWUEeco estimates: comparison of the two estimates | Medium |
| Assumptions of negligible *S* and *G* for iWUEeco: overestimation of iWUEeco | No impact or low as assumed constant over time |

**Figure captions**

**Fig. 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 in Supporting Information). (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 (Δ*c*a) over the same period. EC1-based and EC2-based ecosystem WUE estimates are for iWUEeco (equation (6); Table S2) and IWUEeco (equation (8); Table S3), respectively. Δ*c*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 gray horizontal dotted line in (b) describes a 1:1 relationship between ΔWUE and Δ*c*a, equivalent to a 1:1 relationship between Δ*c*i and Δ*c*a. Significant differences between ΔWUE/Δ*c*a estimates and the 1:1 line (asterisks in colour) and between the different groups of ΔWUE/Δ*c*a (black lines and asterisks) are also indicated (Student-t or Wilcoxon tests; p < 0.05, \*, p < 0.01, \*\* and p < 0.001, \*\*\*).

**Fig. 2** Example of the effect of increasing *c*a over the range of 285-400 ppm on Δ13C (a) and iWUEleaf (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, (3) *c*a - *c*i = 85.5 ppm. The values used for the Δ13C calculations were *a* = 4.4‰, = 27‰, *b* = 30‰, *f* = 12‰ and Γ\* = 43 ppm (*Tair* = 25°C and *Patm* = 98.716 kPa). The numbers on the right indicate the respective trend slopes in ‰ increase of Δ13C ppm-1 increase of *c*a (a) and in µmol mol-1 increase of iWUEleaf ppm-1 increase of *c*a (b). The asterisk denotes the significance of the trend (p < 0.001, \*\*\*).

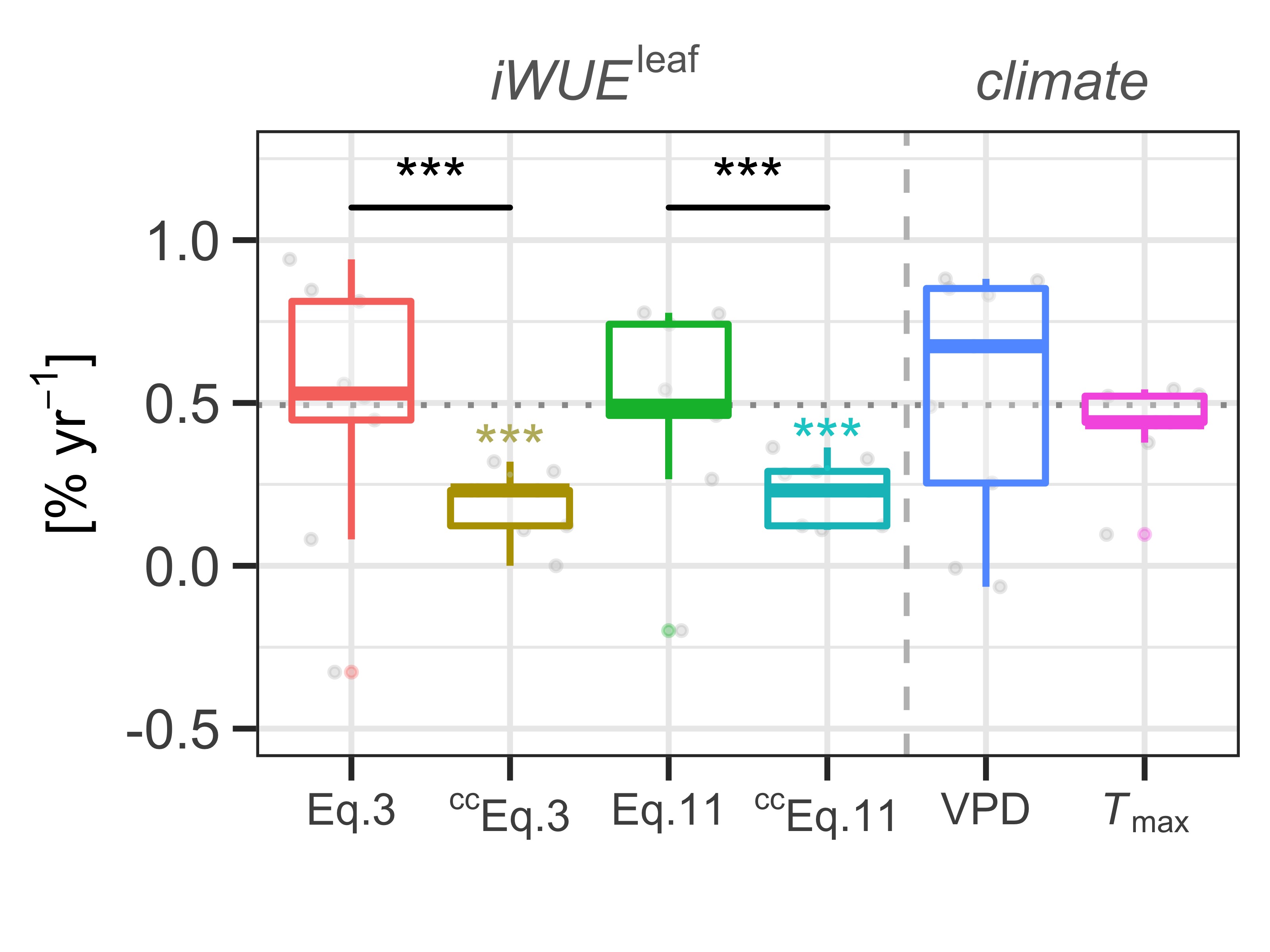
**Fig. 3** Annual changes in tree-ring-based iWUEleaf for raw and climate-corrected (cc) series and in climate drivers (i.e. VPD and *T*max) for the selected 9 tree-ring sites available in the ITRDB network with strong influence from evaporative demand (see Table S5). The Sen slopes of the percentage of changes relative to the site mean (% yr-1) over 1992-2011 are presented. iWUEleaf trends inferred from the simple discrimination model (Eq.3), and that including the photorespiration term only (Eq.11) are compared. The values used for calculations were *a* = 4.4‰, = 27‰, *b* = 30‰, *f* = 12‰, *d* = 2.1‰. Γ\* was calculated as a function of temperature following Bernacchi et al. (2001). The gray horizontal dotted line is the Sen slope of the percentages of changes in *c*a relative to the site mean (% yr-1) over 1992-2011 calculated using direct in-situ measurements from Scripps ([http://scrippsco2.ucsd.edu/data/atmospheric\_co2/primary\_mlo\_co2\_record](http://scrippsco2.ucsd.edu/data/atmospheric_co2/primary_mlo_co2_record;l); last update on 12 December 2018). Summer mean *T*max (°C) and VPD (kPa) were estimated with the CRUTS4.01 gridded dataset (Harris et al., 2014). Significant differences between ΔWUE/Δ*c*a estimates and the 1:1 line (asterisks in colour) and between the different groups of ΔWUE/Δ*c*a (black lines and asterisks) are also indicated (Student-t or Wilcoxon tests; p < 0.001, \*\*\*).

**Fig. 4** Annual changes in WUEeco, IWUEeco, iWUEeco, 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 (% yr-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 (% yr–1) over 1992-2011 calculated using direct in-situ measurements from Scripps ([http://scrippsco2.ucsd.edu/data/atmospheric\_co2/primary\_mlo\_co2\_record](http://scrippsco2.ucsd.edu/data/atmospheric_co2/primary_mlo_co2_record;l); 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 or Wilcoxon tests; p < 0.05, \*).

A close up of a map

Description automatically generated





A close up of a map

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