

# Linking variation in intrinsic water-use efficiency to isohydricity: a comparison at multiple spatiotemporal scales

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## Summary

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- Species-specific responses of plant intrinsic water-use efficiency (iWUE) to multiple environmental drivers associated with climate change, including soil moisture ( $\theta$ ), vapor pressure deficit ( $D$ ), and atmospheric  $\text{CO}_2$  concentration ( $c_a$ ), are poorly understood.
- We assessed how the iWUE and growth of several species of deciduous trees that span a gradient of isohydric to anisohydric water-use strategies respond to key environmental drivers ( $\theta$ ,  $D$  and  $c_a$ ). iWUE was calculated for individual tree species using leaf-level gas exchange and tree-ring  $\delta^{13}\text{C}$  in wood measurements, and for the whole forest using the eddy covariance method.
- The iWUE of the isohydric species was generally more sensitive to environmental change than the anisohydric species was, and increased significantly with rising  $D$  during the periods of water stress. At longer timescales, the influence of  $c_a$  was pronounced for isohydric tulip poplar but not for others.
- Trees' physiological responses to changing environmental drivers can be interpreted differently depending on the observational scale. Care should be also taken in interpreting observed or modeled trends in iWUE that do not explicitly account for the influence of  $D$ .

## Introduction

Terrestrial plants mitigate global warming by removing atmospheric  $\text{CO}_2$  through photosynthesis (Beer *et al.*, 2010). However, because  $\text{CO}_2$  enters the plant through the same pathway by which water exits (i.e. stomata), plants lose water in the process. Thus, it is important to learn how climate change, characterized by increasing atmospheric  $\text{CO}_2$  concentration ( $c_a$ ) and more severe and frequent drought and high-temperature events, influences the trade-off between carbon (C) uptake and water loss (Nepstad *et al.*, 2002; Schar *et al.*, 2004; Salinger, 2005; Breda *et al.*, 2006), which is often represented as the plant's intrinsic water-use efficiency (iWUE = C assimilation rate ( $A$ )/stomatal conductance ( $g_s$ ; Beer *et al.*, 2009; Medlyn *et al.*, 2017).

Previous work suggests that iWUE is sensitive to multiple climate drivers, especially  $c_a$  and water availability. The consensus view, based on previous observational and modeling studies, is that iWUE will increase with increasing  $c_a$  because plants will be able to close stomata (to minimize water loss) without reducing C uptake (Silva *et al.*, 2009, 2016; Brienen *et al.*, 2011; Maseyk *et al.*, 2011; Nock *et al.*, 2011; Battipaglia *et al.*, 2013; Keenan *et al.*, 2013; Frank *et al.*, 2015; van der Sleen *et al.*, 2015).

Drought could also increase iWUE if plants close their stomata to reduce water stress but  $A$  is not reduced by a comparable fraction. Drought-induced increases in iWUE are commonly reported (Ponton *et al.*, 2002; Beer *et al.*, 2009; Maseyk *et al.*, 2011; Linares & Camarero, 2012; Vickers *et al.*, 2012; Battipaglia *et al.*, 2014; Novick *et al.*, 2015; Hentschel *et al.*, 2016; Malone *et al.*, 2016), and relate to the hyperbolic (i.e. saturating) relationship between  $A$  and intercellular  $\text{CO}_2$  concentration ( $c_i$ ; Farquhar *et al.*, 1980); in other words, as stomata close linearly during drought,  $A$  does not always decrease linearly, resulting in increases in iWUE. However, whether changes in iWUE during drought are driven by soil moisture ( $\theta$ ) or atmospheric water demand (i.e. vapor pressure deficit;  $D$ ) is unknown, as both drivers can influence  $g_s$  by changing leaf turgor pressure (Buckley, 2017). The impact of  $D$  on drought responses is often overlooked when compared with the role of  $\theta$  (Novick *et al.*, 2016; Konings *et al.*, 2017; Rigden & Salvucci, 2017), despite recent work suggesting that  $D$  can alter iWUE – independent of changes in  $\theta$  – across multiple ecosystem types (Q. Zhang *et al.*, unpublished). Given that  $D$  and  $\theta$  will be affected differently by climate change (Novick *et al.*, 2016; Ficklin & Novick, 2017), disentangling the effects of  $D$  and  $\theta$  on iWUE is critical for understanding trees'

physiological responses to our changing climate (Williams *et al.*, 2013).

It is also unknown whether iWUE variation is linked to species-specific water-use strategies. Stomatal conductance is an important regulator of iWUE (Tang *et al.*, 2014) and is the basis for a widely accepted framework that classifies trees' hydraulic strategies along the spectrum of isohydry to anisohydry (Tardieu & Simonneau, 1998; Choat *et al.*, 2012; Manzoni *et al.*, 2013; Klein *et al.*, 2014; Martinez-Vilalta *et al.*, 2014; Skelton *et al.*, 2015; Meinzer *et al.*, 2016). According to the framework, species that are more isohydric regulate leaf water potential ( $\Psi_L$ ) within a narrow range by closing stomata to maintain a minimum midday  $\Psi_L$  as water stress progresses, whereas species that are more anisohydric allow  $\Psi_L$  to decrease under water stress (Tyree & Sperry, 1988; McDowell *et al.*, 2008; Choat *et al.*, 2012; Manzoni *et al.*, 2013; Martinez-Vilalta *et al.*, 2014; Martinez-Vilalta & Garcia-Forner, 2017). As a result, isohydric species that maintain relatively constant  $\Psi_L$  by closing their stomata rapidly as drought stress develops are likely to benefit more from a drought-driven increase in iWUE, as isohydric species could achieve cost-efficient C uptake and avoid a potential risk of hydraulic failure.

Even if the controls on iWUE are well characterized, the variable approaches used to quantify iWUE – from the leaf to the ecosystem, and from hourly to seasonal scales – may not always lead to the same conclusions about what controls iWUE. Leaf gas exchange measurements and  $\delta^{13}\text{C}$  analysis of tree-rings enable the estimation of iWUE for individual trees. The former method can directly measure C and water exchange at the leaf level, and at short temporal scale (i.e. daily) if observations are made frequently. However, this approach is labor intensive and requires accessing leaves of tall trees. Tree-ring methods exploit the fact that the  $\delta^{13}\text{C}$  of assimilated C is affected by a plant's photosynthetic capacity and stomatal regulation (Farquhar *et al.*, 1989). Because these values are preserved in the rings, estimates of iWUE from tree cores can provide a time-integrated measure of iWUE over the course of the growing season (Battipaglia *et al.*, 2013; Belmecheri *et al.*, 2014; Frank *et al.*, 2015). However, the tree-ring approach is less useful for detecting short-term physiological changes that occur during a growing season in response to water stress. A third method for calculating iWUE is eddy covariance, where high temporal resolution data can be leveraged to estimate an ecosystem-scale analogue of iWUE:  $\text{iWUE}^* = \text{gross primary productivity (GPP)}/\text{surface conductance}$ . While this approach cannot resolve species-specific leaf- or tree-scale dynamics, and cannot partition transpiration from evapotranspiration (Keenan *et al.*, 2013), the method is effective for coupling high temporal resolution iWUE and meteorological data, which can facilitate an improved understanding of environmental controls.

With a few exceptions (e.g. Monson *et al.*, 2010; Scartazza *et al.*, 2014; Guerrieri *et al.*, 2016; Medlyn *et al.*, 2017), most previous work has evaluated iWUE at a single spatial or temporal scale (e.g. Hietz *et al.*, 2005; Cernusak *et al.*, 2007; Brienen *et al.*, 2011; Altieri *et al.*, 2015). Comparison of iWUE between different scales is important since some factors may influence iWUE

quantification only at one scale or vary in relative importance across scales. For example, leaf-scale iWUE is mainly regulated by  $g_s$  and biochemical processes in the chloroplasts. Ecosystem-scale iWUE\* is also affected by the evaporation and canopy structure (Tang *et al.*, 2014), and tree-scale iWUE is affected by the allocation of structural carbohydrates (i.e. different C investment to different tree organs) and the formation, storage and utilization of nonstructural carbohydrates (NSCs) to regulate plant metabolism (Hartmann & Trumbore, 2016). Rigorous assessments of the scale dependencies of iWUE will not only provide insight into trees' physiological responses to climate change, but also can improve the prediction of ecological models by resolving the issues that arise from the transferring of ecological information across scales.

In this study, we assessed how iWUE of deciduous trees growing in the Eastern US responded differently to key environmental drivers ( $\theta$ ,  $D$  and  $c_i$ ) across spatial (leaf, tree, and ecosystem) and temporal (hourly, daily to weekly, and annual) scales and across a spectrum of isohydricity. Three tree species known to represent, collectively, very isohydric (tulip poplar), intermediate (sugar maple), and very anisohydric (white oak) behavior were selected (Roman *et al.*, 2015; Yi *et al.*, 2017). We hypothesized that the iWUE of isohydric species will be more responsive to changes in water availability (i.e.  $\theta$  and  $D$ ), reflecting the expectation that, in isohydric plants, as  $g_s$  declines  $c_i$  will also decline but  $A$  will decrease more slowly due to the hyperbolic shape of the  $A$ - $c_i$  curve. In reality,  $A$  and  $g_s$  may be further decoupled by drought-driven changes in mesophyll conductance (which could affect  $c_i$  independently of changes in  $g_s$ ; Grassi & Magnani, 2005; Flexas *et al.*, 2008, 2012), as well as drought-driven declines in photosynthetic capacity (Tezara *et al.*, 1999; Chaves *et al.*, 2003; Grassi & Magnani, 2005). Our hypothesis of greater increases in iWUE for isohydric species is therefore predicated on the assumption that it would be beneficial for these species to have co-evolved traits that limit drought-driven declines in C uptake from nonstomatal limitations, which could be pronounced for species that are more anisohydric that experience significant declines in  $\Psi_L$  during drought. We tested this hypothesis by comparing iWUE estimated using leaf gas exchange (leaf scale) and  $\delta^{13}\text{C}$  analysis of tree-ring cellulose (tree scale). Finally, we assessed how variation of iWUE relates to the dynamics of primary productivity and growth by estimating  $A$ , basal area increment (BAI), and GPP at the leaf-, tree-, and ecosystem-scales respectively.

## Materials and Methods

### Study site

The study site is located inside the footprint of the Morgan–Monroe State Forest (MMSF) Ameriflux tower (US-MMS) in south-central Indiana, USA (39°19'N 86°25'W, 275 m asl). The MMSF is a secondary mixed hardwood composed of sugar maple (*Acer saccharum* Marshall), tulip poplar (*Liriodendron tulipifera* L.), sassafras (*Sassafras albidum* (Nutt.) Nees) and oaks (*Quercus* spp.), which represent nearly 75% of the total basal

area (Schmid *et al.*, 2000). Mean age of the trees is *c.* 90 yr, with a mean height of 27 m (Roman *et al.*, 2015). The soil type is Typic Dystrochrepts dominated by the Berks–Weikert complex, defined as well-drained silt-loam (Dragoni *et al.*, 2011). The study period includes 2012, a year marked by an extreme drought event that affected much of the Midwestern US. This event was one of the ‘costliest . . . and most widespread natural disasters in US history’ (Mallya *et al.*, 2013). The soil moisture deficits and high temperatures associated with this event reduced C and water exchange at MMSF (see Supporting Information Fig. S1) and restricted the growth of many species (Roman *et al.*, 2015; Yi *et al.*, 2017).

In previous work from the site, a diagnostic framework was developed to classify isohydric and anisohydric behaviors of the canopy dominants (Roman *et al.*, 2015; Yi *et al.*, 2017) based on the response of  $g_s$  to  $D$  such that:

$$g_s = K(\Psi_S - \Psi_L)/D = K(\Delta\Psi)/D \quad \text{Eqn 1}$$

where  $K$  is the whole-plant hydraulic conductance,  $\Psi_S$  is the soil water potential, and  $\Delta\Psi$  is the difference between  $\Psi_S$  and  $\Psi_L$ . The contribution of gravitational head losses to  $\Delta\Psi$ , which is relatively constant over weekly to monthly timescales, is neglected. Using this relationship, degree of isohydricity was characterized for dominant species under water stress conditions as follows:

- Isohydric behavior:  $\Delta\Psi$  decreases rapidly during the period of water stress as  $\Psi_L$  remains relatively constant, reflecting declines in the magnitude of  $g_s$  as well as its sensitivity to  $D$  relative to the well-watered period;
- Anisohydric behavior:  $\Psi_L$  declines, and  $\Delta\Psi$  remains constant or even increases, for a longer portion of the period of water stress; as a result,  $g_s$  and its sensitivity to  $D$  are sustained or increased relative to the well-watered period.

Based on this framework, tulip poplar, sugar maple and white oak (*Quercus alba* L.) growing in the MMSF were classified as very isohydric, intermediate, and very anisohydric species respectively – see Fig. S2, and Roman *et al.* (2015) and Yi *et al.* (2017) for more details. Differences in rooting depth and soil moisture access are important to consider when classifying species along the isohydricity spectrum; specifically, higher  $g_s$  under water stress may be due to deeper rooting depth instead of anisohydric traits (Hochberg *et al.*, 2018). However, in our site, measured pre-dawn  $\Psi_L$  (often assumed to be an indicator of the total plant-available soil moisture across the rooting zone) does not indicate that white oak growing in MMSF accessed deeper pools of water compared with the most isohydric tulip poplar (see Table S1).

### Eddy covariance measurement and edaphic data

The 46 m flux tower has been continuously recording fluxes of CO<sub>2</sub>, water, and energy at a frequency of 10 Hz since 1998 using a three-dimensional sonic anemometer (CSAT-3; Campbell Scientific, Logan, UT, USA) and a closed-path infrared gas analyzer (LI-7000; Li-Cor, Lincoln, NE, USA). For this study, we used data including  $D$  and  $c_a$  collected at a height of 46 m above the ground from 1999 to 2013 (see Fig. S3). Post-processing

protocols are explained in detail elsewhere (Dragoni *et al.*, 2011; Roman *et al.*, 2015). To fill hourly net ecosystem exchange (NEE) time-series gaps and to partition NEE into GPP and ecosystem respiration (RE), simple parametric models that link soil temperature to RE and photosynthetic photon flux density to GPP were applied to the NEE records, such that  $NEE = RE - GPP$  (Dragoni *et al.*, 2011).

Volumetric water content ( $\theta$ ) in the first 30 cm of the soil was monitored at four different locations in the footprint of the flux tower using time-domain reflectometers (TDRs; CS615 and CS616; Campbell Scientific). The data were then scaled using gravimetric soil samples collected weekly at the locations of TDR monitoring and a site-wide average  $\theta$  was calculated (Roman *et al.*, 2015).

### Tree-ring chronologies

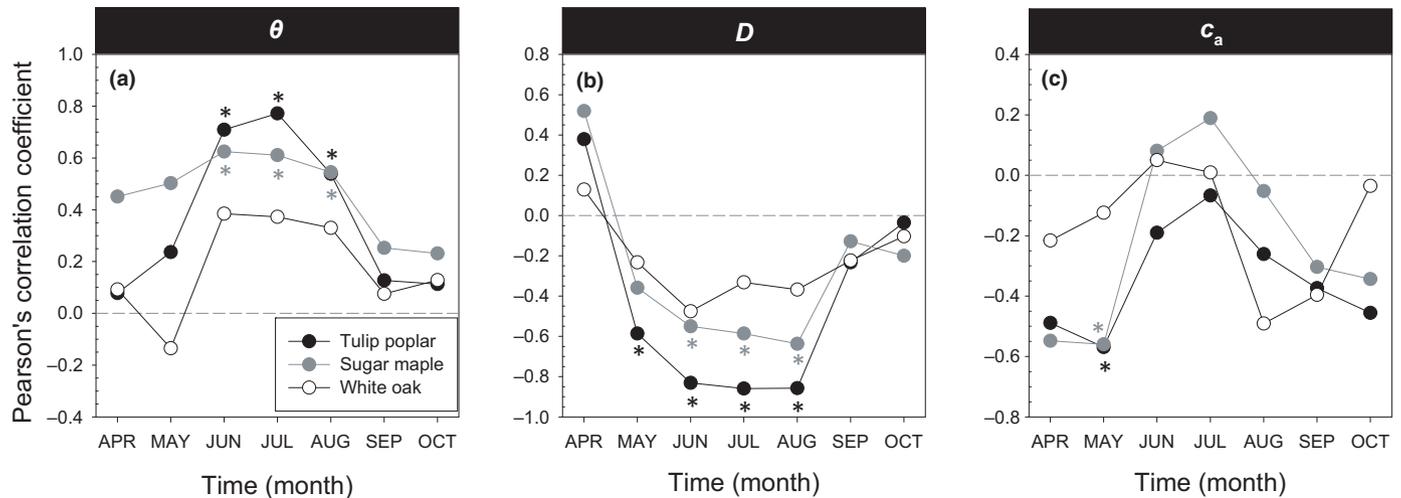
Tree cores were sampled for canopy-dominant tulip poplars, sugar maples, and white oak (*c.* 10 replicates per species; Table 1) using a 5 mm diameter increment borer at breast height, while ensuring penetration of the pith. Tree cores were air-dried, mounted, and sanded until cell structure was clearly visible. The growth-rings of each sample were measured using a measuring stage (Velmex, Bloomfield, NY, USA) and the MEASUREJ2X program (VoorTech Consulting, Holderness, NH, USA). The tree-ring chronologies were statistically cross-dated (Table 1) using the COFECHA program (Holmes, 1983). Annual BAI from 1999 to 2012 was calculated from the four randomly-selected tree-ring chronologies of each species (see Fig. S4). To evaluate how the seasonality of each environmental driver influences radial growth, we identified the months between April and October that show a significant correlation between mean monthly  $\theta$ ,  $D$  or  $c_a$  and annual BAI (1999–2012) by quantifying the Pearson correlation coefficient (Fig. 1).

### Leaf gas exchange measurements

In-situ rates of  $g_s$ ,  $A$ , and transpiration rate ( $E$ ) were measured during the growing seasons of 2011 to 2013 using a portable photosynthesis system (LI-6400XT; Li-Cor) with leaves accessed using a 24 m boom lift. The measurements were performed weekly for five sunlit canopy leaves per tree and three trees per species (note that these trees are different from trees that were cored). The environmental conditions inside the leaf chamber were set to match ambient conditions as described in Roman *et al.* (2015). All measurements were averaged for each species and each day.

**Table 1** Tree-ring statistics for each species

Species	No. of trees (cores)	Average age	Interseries correlation
Tulip poplar	10 (20)	65	0.620
Sugar maple	9 (17)	88	0.494
White oak	11 (21)	69	0.575



**Fig. 1** Pearson's correlation coefficient (Pearson's  $r$ ) between environmental drivers (a) mean monthly soil moisture content ( $\theta$ ), (b) vapor pressure deficit ( $D$ ), or (c) atmospheric  $\text{CO}_2$  concentration ( $c_a$ ) and annual radial growth (basal area increment, BAI) inferred from tree-ring samples. Pearson's  $r$  closer to +1 or -1 indicates stronger positive or negative relationship respectively between environmental drivers and BAI. Asterisks on the data points represent the significant linear relationship between environmental drivers and BAI: \*,  $P < 0.05$ .

### Intrinsic WUE at different scales

The  $i\text{WUE}_L$  at the leaf ( $i\text{WUE}_L$ ), tree ( $i\text{WUE}_T$ ), and ecosystem scales ( $i\text{WUE}^*$ ) were computed as follows:

$$i\text{WUE}_L = A/g_s \quad \text{Eqn 2}$$

$$i\text{WUE}_T = (c_a - c_i)/1.6 \quad \text{Eqn 3}$$

$$i\text{WUE}^* = \text{GPP}/\text{ET} \times (D/P_a) \quad \text{Eqn 4}$$

where ET is evapotranspiration rate and  $P_a$  is atmospheric pressure. The relationship between  $c_i$  and  $c_a$  for the estimation of  $i\text{WUE}_T$  can be obtained from the following two equations for C isotope discrimination ( $\Delta$ ):

$$\Delta = a + (b - a)c_i/c_a \quad \text{Eqn 5}$$

$$\Delta = (\delta^{13}\text{C}_{\text{air}} - \delta^{13}\text{C}_{\text{plant}})/(1 + \delta^{13}\text{C}_{\text{plant}}/1000) \quad \text{Eqn 6}$$

where  $a$  is a constant of fractionation during  $\text{CO}_2$  diffusion through the stomata (4.4‰; O'Leary, 1981),  $b$  is a constant of fractionation by ribulose 1,5-bisphosphate carboxylase (27‰; Farquhar & Richards, 1984),  $\delta^{13}\text{C}_{\text{air}}$  is the atmospheric  $\delta^{13}\text{C}$  obtained from Mauna Loa Observatory (Keeling *et al.*, 2005), and  $\delta^{13}\text{C}_{\text{plant}}$  is the  $\delta^{13}\text{C}$  from the tree-ring samples. For  $\delta^{13}\text{C}_{\text{plant}}$ , only the latewood portion of annual tree rings was used, as previous work suggests that latewood, but not earlywood, reliably reflects the climate signal of the current year (Lipp *et al.*, 1991; Livingston & Spittlehouse, 1996; Battipaglia *et al.*, 2009). This assumption is supported by the fact that the annual ring-width increment was best correlated to the variation of  $\theta$  and  $D$  during the period from June to August in MMSF (Fig. 1). To collect latewood portion of annual rings, cores were dissected using a scalpel under a microscope. Earlywood and latewood were easily distinguishable for white oak (ring porous), but not for tulip poplar and sugar maple (diffuse porous). In the case of diffuse-porous species, we only used the last one-third of annual

rings, assuming that contribution of earlywood to that portion is negligible. The latewood samples of two cores originating from a single tree were pooled as a single sample for the  $\delta^{13}\text{C}$  analysis. For each sample,  $\alpha$ -cellulose was extracted using a modified Soxhlet method (Loader *et al.*, 1997), and  $\delta^{13}\text{C}_{\text{plant}}$  was measured on the  $\text{CO}_2$  produced by  $\alpha$ -cellulose combustion using an elemental analyzer (ECS4010; Costech, Valencia, CA, USA) coupled to an isotope-ratio mass spectrometer (Finnigan DELTAplusXP; ThermoFisher Scientific, Waltham, MA, USA). The  $i\text{WUE}_L$  was computed using the output of gas exchange measurements, and the  $i\text{WUE}^*$  from 2011 to 2013 was estimated from Eqn 4 using mean hourly GPP, ET,  $D$  and  $P_a$  inferred from the flux tower.

### Statistical analyses

The species-specific response of  $i\text{WUE}$ ,  $A$ , and GPP to each environmental driver (i.e.  $\theta$ ,  $D$ , and  $c_a$ ) was assessed. For the  $i\text{WUE}$  analyses, we considered environmental drivers collected during the late growing season (June–August) when latewood (the basis for the  $i\text{WUE}_T$  data) forms (see Fig. S5). For  $A$  and GPP, we considered observations from the entire growing season. An initial assessment of the response of each biophysical variable to each environmental driver was accomplished by partitioning the data into discrete bins spanning a range of the driver of interest and using one-way analysis of variance and Tukey post-hoc test using SPSS STATISTICS (v.24). All statistical tests were performed at a significance level of 0.05. For context, we also evaluated the influence of the environmental drivers on water loss at the leaf ( $g_s$  and  $E$ ) and ecosystem (canopy conductance ( $g_c$ ) and ET) scales using an analogous approach (see Figs S6, S7). The relationship between species-specific BAI (averaged across all trees) and the environmental drivers was also assessed using linear regression, where the environmental variables were averaged over the course of the entire growing season (April–October).

Finally, we evaluated the relative influence of each environmental driver on iWUE across different scales and species using multiple linear regression. For this, iWUE was set as a dependent variable and environmental drivers were set as independent variables. We compared standardized coefficients ( $\beta$ ) associated with the three independent variables to assess their relative influence on iWUE. The standardized coefficients are the estimates resulting from a multiple regression analysis and refer to how many standard deviations the iWUE will change per standard deviation increase in each environmental driver. This approach is advantageous because we can directly compare  $\beta$  of  $\theta$ ,  $D$ , and  $c_a$  without concern for their variable units. Usage of regression analysis was validated by identifying normality, linearity between dependent and independent variables, homoscedasticity of residuals, and absence of multicollinearity (data not presented). The temporal scales were defined by the data collection frequency of each measurement: leaf gas exchange measurement (leaf scale) represents daily to weekly scales,  $\delta^{13}\text{C}$  using tree-rings (tree scale) represents seasonal to annual scales, and eddy covariance (ecosystem scale) represents an hourly scale.

## Results

### iWUE at different scales

The relationships between iWUE and environmental drivers were different depending on the species and spatiotemporal scales (Fig. 2). In general, tulip poplar was more responsive to the change in environmental drivers, whereas the responses of other species were static or not significant (except iWUE vs  $D$  at the leaf scale; Fig. 2d).

In the case of the relationship between iWUE and  $\theta$ , tulip poplar was the only species showing a significant negative relationship ( $P < 0.05$ ) at both leaf (daily to weekly) and tree (seasonal to annual) scales (Fig. 2a,b). The highest iWUE<sub>L</sub> of tulip poplar was  $99.6 \mu\text{mol C mol}^{-1} \text{H}_2\text{O}$  when  $\theta$  was between  $0.1$  and  $0.2 \text{ m}^3 \text{ m}^{-3}$ , 47% higher than the iWUE<sub>L</sub> when  $\theta$  was high (to  $0.5 \text{ m}^3 \text{ m}^{-3}$ ). The change of iWUE<sub>T</sub> with  $\theta$  for tulip poplar was less pronounced. The highest iWUE<sub>T</sub> of tulip poplar was  $118.4 \mu\text{mol C mol}^{-1} \text{H}_2\text{O}$  when  $\theta$  was between  $0.1$  and  $0.2 \text{ m}^3 \text{ m}^{-3}$ , 14% higher than the iWUE<sub>T</sub> when  $\theta$  was greater than  $0.3 \text{ m}^3 \text{ m}^{-3}$ . The iWUE of sugar maple increased as  $\theta$  declined at both scales, but without statistical significance ( $P > 0.05$ ). The highest iWUE<sub>L</sub> and iWUE<sub>T</sub> of sugar maple were  $100.3 \mu\text{mol C mol}^{-1} \text{H}_2\text{O}$  and  $122.7 \mu\text{mol C mol}^{-1} \text{H}_2\text{O}$  respectively, which were up to 14% and 10% respectively higher than the iWUE observed at higher  $\theta$ . Variations of iWUE<sub>L</sub> and iWUE<sub>T</sub> with varying  $\theta$  were modest and statistically insignificant for the white oak ( $P > 0.05$ ).

The iWUE<sub>L</sub> of all species increased with increasing  $D$  ( $P < 0.05$ , Fig. 2d). The iWUE<sub>L</sub> of tulip poplar, sugar maple, and white oak increased by 160%, 190% and 80% respectively as  $D$  increased from 0 to 5 kPa. The pattern of iWUE<sub>T</sub> was similar to that of iWUE<sub>L</sub>, but the change in iWUE<sub>T</sub> was smaller than in iWUE<sub>L</sub> (Fig. 2e). The iWUE<sub>T</sub> of tulip poplar, sugar maple, and white oak increased by 20%, 10%, and 0% respectively as  $D$

increased from 0.5 to 2 kPa (Fig. 2e), which is comparable to 50%, 80%, and 20% respectively higher iWUE<sub>L</sub> as  $D$  increased from 0 to 2 kPa (Fig. 2d). Furthermore, in contrast to the results of iWUE<sub>L</sub>, iWUE<sub>T</sub> increased with increasing  $D$  only for the highly isohydric tulip poplar ( $P < 0.05$ ).

The response of iWUE to the variation of  $c_a$  was not consistent across the scales compared with the other environmental drivers (Fig. 2). The iWUE<sub>L</sub> for all species was not significantly responsive to the variation of  $c_a$  ( $P > 0.05$ ). The iWUE<sub>T</sub> of tulip poplar increased by 18% as  $c_a$  increased from 350 to  $425 \mu\text{mol mol}^{-1}$  ( $P < 0.05$ ), whereas the iWUE<sub>T</sub> of other species did not vary significantly over this range of  $c_a$  ( $P > 0.05$ , Fig. 2h).

The responses of iWUE\* to  $\theta$  or  $D$  were similar to the leaf- and tree-scale dynamics of tulip poplar (Fig. 2). Furthermore, it was notable that the magnitude of iWUE\* was always lower than the other scales. The highest iWUE\* was  $55.4 \mu\text{mol C mol}^{-1} \text{H}_2\text{O}$  when  $\theta$  was between  $0.1$  and  $0.2 \text{ m}^3 \text{ m}^{-3}$ , which was 26% higher than iWUE\* observed when  $\theta$  was between  $0.4$  and  $0.5 \text{ m}^3 \text{ m}^{-3}$  ( $P < 0.05$ , Fig. 2c), whereas iWUE\* increased from  $34.6 \mu\text{mol C mol}^{-1} \text{H}_2\text{O}$  by 141% as  $D$  increased from 0 to 5 kPa ( $P < 0.05$ , Fig. 2f). The response of iWUE\* to  $c_a$  was different from the other scales; specifically, it was decreased by 22% as  $c_a$  increased from 350 to  $430 \mu\text{mol mol}^{-1}$  ( $P < 0.05$ , Fig. 2i).

### Relative influence of environmental drivers on iWUE

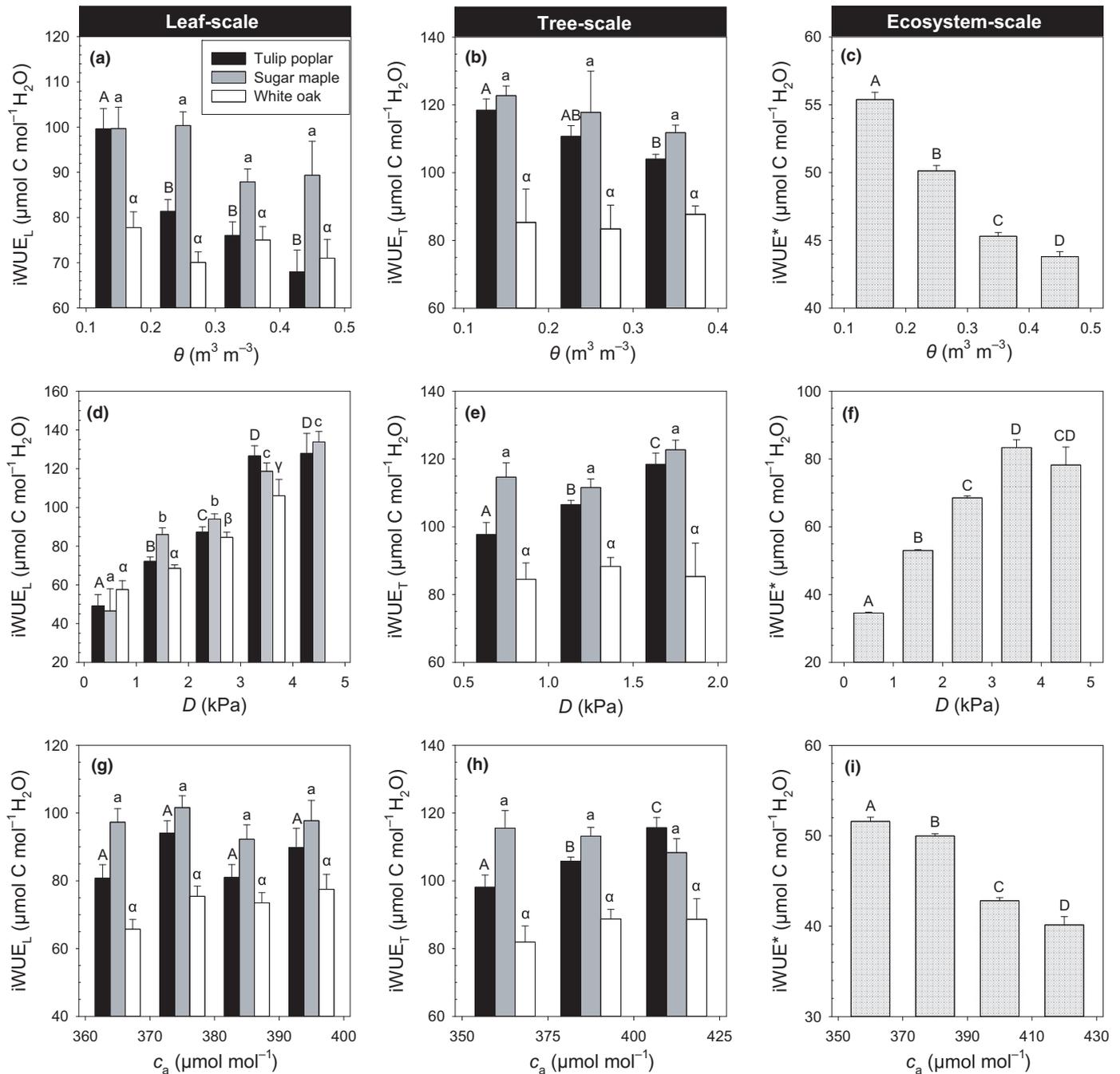
The iWUE was sensitive to different environmental drivers depending on tree species (Fig. 3). Moreover, the relative influence of these drivers could be interpreted differently depending on the observational scale. The iWUE\* was most sensitive to the variation of  $D$  in a positive direction, accounting for 86% of the total influence of three drivers, whereas  $\theta$  and  $c_a$  only accounted for 5% and 9% respectively (Fig. 3). Despite the weight on  $D$ , all environmental drivers were significantly influential on the iWUE\* ( $P < 0.05$ ).

The iWUE<sub>L</sub> was most sensitive to the variation of  $D$  for all species, accounting for 64%, 76% and 64% of the total influence on iWUE<sub>L</sub> of tulip poplar, sugar maple, and white oak respectively (Fig. 3). The relative influence of  $c_a$  on iWUE<sub>L</sub> was larger than on iWUE\*, accounting for 22%, 24%, and 30% of total influence of tulip poplar, sugar maple, and white oak respectively. The influence of  $\theta$  on iWUE<sub>L</sub> was the smallest among the environmental drivers. Although the iWUE<sub>L</sub> of tulip poplar was observed to increase with decreasing  $\theta$  ( $-14\%$ ,  $P < 0.05$ ), the influence of  $\theta$  on the iWUE<sub>L</sub> of sugar maple and white oak was negligible ( $P > 0.05$ ).

The iWUE<sub>T</sub> of tulip poplar was most sensitive to the variation of  $c_a$  (57%,  $P < 0.05$ ), followed by  $D$  (33%,  $P < 0.05$ ), but not sensitive to  $\theta$  ( $P > 0.05$ ). In the case of sugar maple and white oak, none of the environmental drivers were significantly influential on their iWUE<sub>T</sub> ( $P > 0.05$ ).

### Carbon gain at different scales

The observed relationships between C gain and environmental drivers were different depending on the species and



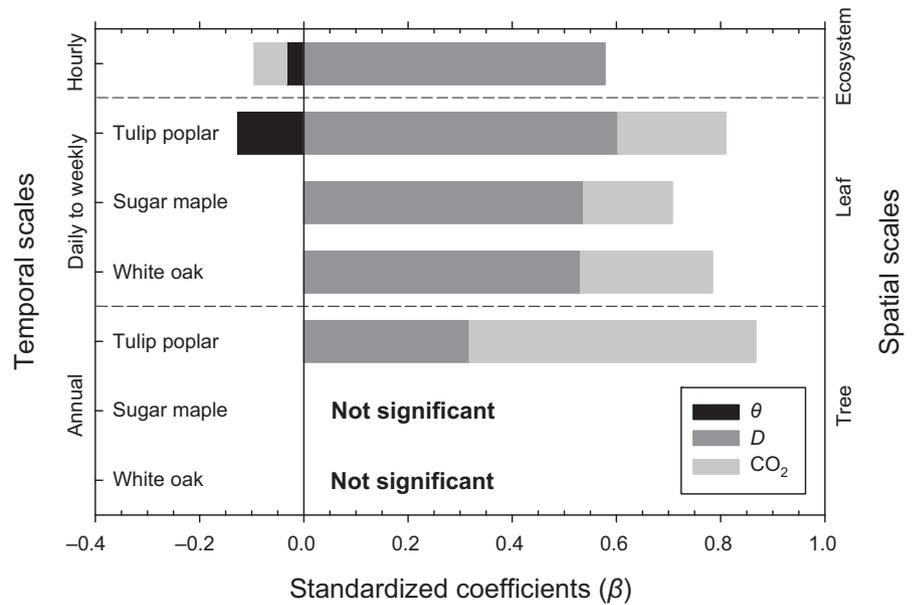
**Fig. 2** Variation of intrinsic water-use efficiency (iWUE) over the range of (a–c) soil moisture contents ( $\theta$ ), (d–f) vapor pressure deficit ( $D$ ), and (g–i) atmospheric  $\text{CO}_2$  concentration ( $c_a$ ) observed at (a, d, g) the leaf, (b, e, h) tree, and (c, f, i) ecosystem scales in Morgan–Monroe State Forest. The iWUEs at the leaf, tree, and ecosystem scales were estimated using leaf gas exchange measurement,  $\delta^{13}\text{C}$  analysis of annual tree rings, and the eddy covariance method respectively. The observation period was the growing seasons from 2011 to 2013 for leaf scale, 1999 to 2012 for tree scale, and 1999 to 2013 for ecosystem scale. Note that measurements at the leaf and ecosystem scales are based on the instant records, whereas the tree-scale measurement is the average of each environmental driver during the late growing season (June–August) of each year. Error bars represent SE of the means (95% confidence). Bars with different characters indicate significant differences in iWUE among the different groups of environmental drivers ( $P < 0.05$ ). Note that interactions between the drivers are not accounted for in this presentation; see Fig. 3 for a discussion of the relative influence of  $\theta$ ,  $D$  and  $c_a$  on iWUE.

spatiotemporal scales (Figs 4,5). In general, the pattern of the relationships was opposite to that of iWUE. Variation of  $A$  (leaf/daily to weekly scale) of tulip poplar and sugar maple were positively related to  $\theta$  (Fig. 4a), negatively to  $D$  (Fig. 4c), and not related to  $c_a$  (Fig. 4e). The  $A$  of white oak was not influenced by any environmental drivers ( $P > 0.05$ ). The  $A$  of tulip poplar and

sugar maple increased up to 34% and 42% respectively with increasing  $\theta$  from the minimum values (Fig. 4a), and the  $A$  of both species decreased up to 47% and 38% respectively with increasing  $D$  from the maximum values (Fig. 4c).

The BAI variation (tree/annual scale) in response to the environmental drivers was different among species (Fig. 5). Overall,

**Fig. 3** Standardized coefficients ( $\beta$ ) of soil moisture contents ( $\theta$ ), vapor pressure deficit ( $D$ ), and atmospheric  $\text{CO}_2$  concentration ( $c_a$ ) for the intrinsic water-use efficiency (iWUE) observed at the different scales in the Morgan–Monroe State Forest. The standard coefficients are the estimates resulting from a multiple regression analysis, and refer to how many SDs an iWUE will change per SD increase in either  $\theta$ ,  $D$  or  $c_a$ . Note that the predictors without statistical significance ( $P > 0.05$ ) are not presented.



the variability of BAI was highest for tulip poplar, ranging from 19.7 to 113.5  $\text{cm}^2 \text{yr}^{-1}$ , whereas the BAI of white oak was generally low and confined in a narrower range from 17.8 to 28.6  $\text{cm}^2 \text{yr}^{-1}$ . The response of sugar maple was intermediate between tulip poplar and white oak (12.4 to 43.2  $\text{cm}^2 \text{yr}^{-1}$ ). The BAI of tulip poplar and sugar maple were positively related to  $\theta$  and negatively to  $D$  ( $P < 0.05$ ); however, no significant relationships were observed from white oak ( $P > 0.05$ ). Unlike the other environmental drivers,  $c_a$  did not influence BAI for any species ( $P > 0.05$ ).

The relationship between GPP (ecosystem/hourly scale) and environmental drivers was similar to the pattern of  $A$  and BAI for tulip poplar (Fig. 4). GPP was positively related to  $\theta$  (Fig. 4b). GPP was highest (19.7  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) when  $\theta$  was between 0.2 and 0.4  $\text{m}^3 \text{m}^{-3}$ , and was reduced by 27% when  $\theta$  decreased to 0.1  $\text{m}^3 \text{m}^{-3}$ . Increase of  $\theta$  beyond 0.4  $\text{m}^3 \text{m}^{-3}$  slightly lowered GPP (5%) when compared with the highest observed GPP ( $P < 0.05$ ). GPP was negatively related to  $D$  when  $D$  was higher than 2 kPa (Fig. 4d). The highest GPP was observed at  $D$  between 1 and 2 kPa (19.9  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and decreased by 58% when  $D$  increased beyond 4 kPa. No directional relationship between GPP and  $c_a$  was observed (Fig. 4f). GPP decreased with  $c_a$  until  $c_a$  reached 410  $\mu\text{mol mol}^{-1}$ , and then increased beyond that  $c_a$  level. The highest GPP was 21.7  $\mu\text{mol m}^{-2} \text{s}^{-1}$  when  $c_a$  was between 350 and 370  $\mu\text{mol mol}^{-1}$ , which was 37% higher than the lowest GPP observed when  $c_a$  was between 390 and 410  $\mu\text{mol mol}^{-1}$ .

#### Relative influence of environmental drivers on C gain

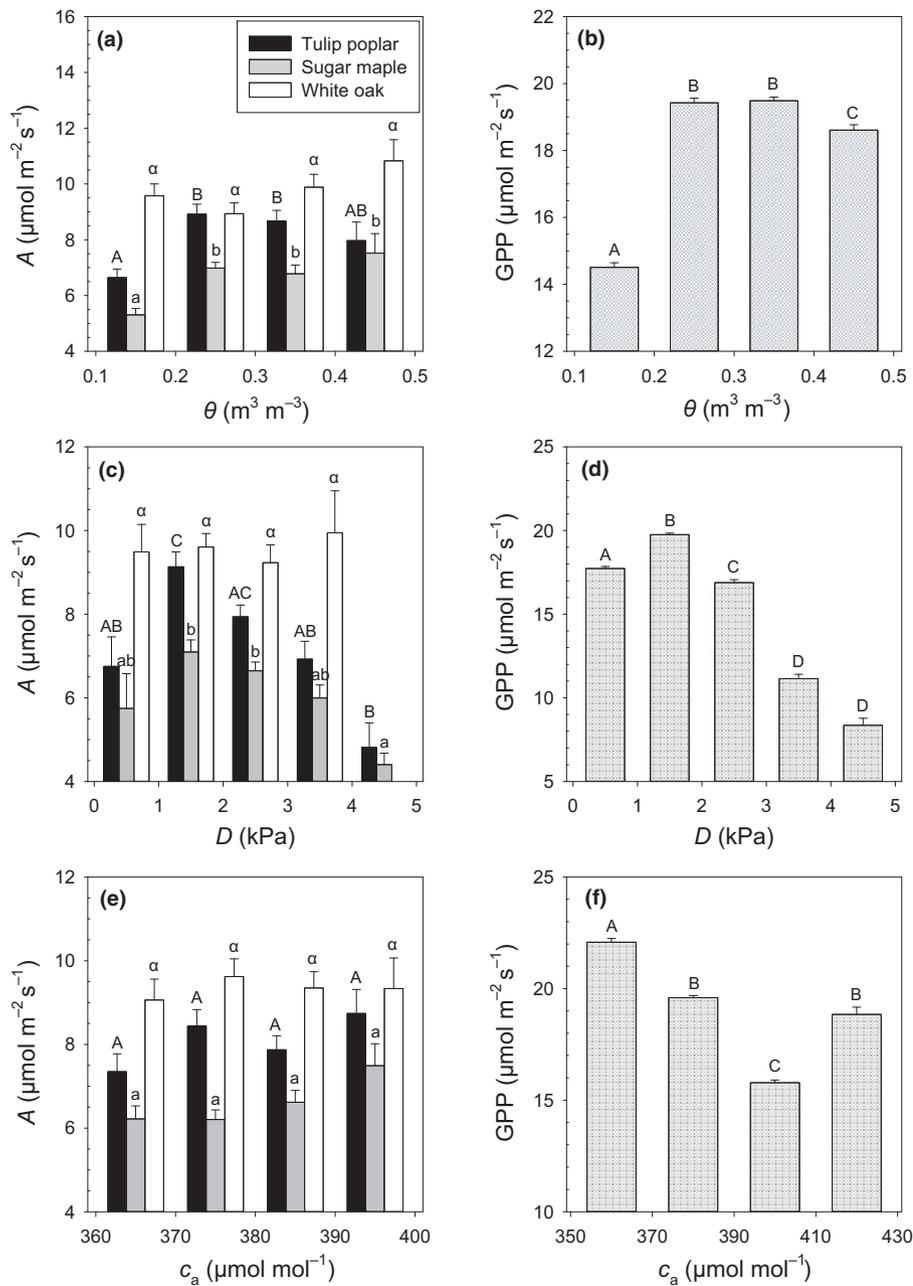
C gain was distinctively influenced by multiple environmental drivers depending on the scales and trees species (Fig. 6), however, the pattern was not similar to that of iWUE (Fig. 3). At the hourly (ecosystem) scale, C gain (i.e. GPP) was most sensitive to the variation of  $c_a$  ( $-60\%$ ,  $P < 0.05$ ), followed by  $\theta$  ( $+31\%$ ) and

then  $D$  ( $-9\%$ ) ( $P < 0.05$ , Fig. 6). At the daily to weekly (leaf) scale, C gain (i.e.  $A$ ) was most sensitive to the variation of  $\theta$  for tulip poplar and sugar maple, accounting for 51% and 62% of the total influence respectively (Fig. 6). The relative influence of  $D$  on  $A$  was larger than that observed at the hourly scale, accounting for 42% and 36% for tulip poplar and sugar maple respectively. The influence of  $c_a$  was negligible for all species ( $P > 0.05$ ), and white oak's  $A$  was not sensitive to any environmental drivers at the daily scale ( $P > 0.05$ ). At the annual (tree) scale, tulip poplar was the only species sensitive to any of the environmental drivers (Fig. 6) and was only sensitive to variation of  $D$  ( $P < 0.05$ ).

#### Discussion

The aim of our study was to compare how the iWUEs of tree species with different isohydricities respond to key environmental drivers at multiple spatiotemporal scales. We found that, for all species, iWUE increased during periods of water stress, primarily reflecting the action of rising  $D$  at relatively short timescales. At longer timescales, the iWUE of isohydric species was more sensitive to variation in environmental drivers than that of anisohydric species was, as hypothesized. Furthermore, the sensitivity of iWUE to variation in  $\theta$  or  $D$  was higher at the leaf scale than at the tree scale. These findings imply that trees respond to environmental changes differently as a reflection not only of their water-use strategies, but also the duration and intensity of environmental changes. As discussed in detail later, they have important implications for using historic observations to diagnose long-term trends in iWUE, and to apply that information to predict future gas exchange dynamics.

We observed that species that were more isohydric tend to increase iWUE when water availability is low (i.e. low  $\theta$  or high  $D$ ), whereas anisohydric species maintain stationary iWUE independent of moisture condition (Fig. 2). Higher iWUE for species

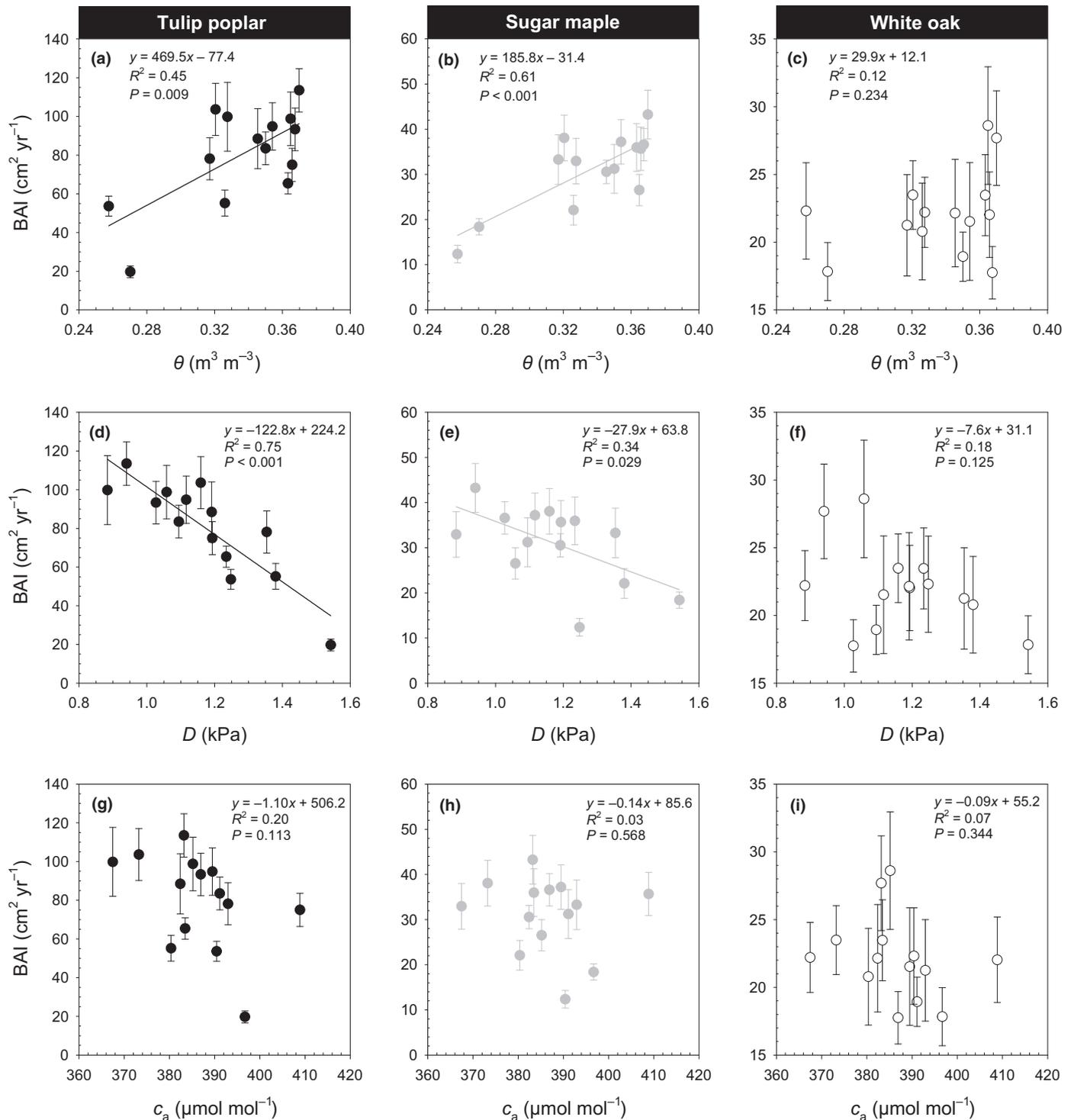


**Fig. 4** Variation of carbon assimilation rate (A) and gross primary production (GPP) over the range of (a, b) soil moisture content ( $\theta$ ), (c, d) vapor pressure deficit (D), and (e, f) atmospheric  $\text{CO}_2$  concentration ( $c_a$ ) observed at (a, c, e) the leaf and (b, d, f) ecosystem scales in the Morgan–Monroe State Forest. Each bar represents mean A or GPP in each group of  $\theta$ , D or  $c_a$  observed during growing seasons (April–October) from 1999 to 2012. Error bars represent SE of the means (95% confidence). Bars with different characters above them indicate a significant difference in A or GPP among the different groups of  $\theta$ , D or  $c_a$  ( $P < 0.05$ ). Note that interactions between the drivers are not accounted for in this presentation; see Fig. 6 for a discussion of the relative influence of  $\theta$ , D and  $c_a$  on A (leaf scale) or GPP (ecosystem scale).

that were more isohydric and/or under drier conditions has been reported previously (Klein *et al.*, 2013; Attia *et al.*, 2015; Renninger *et al.*, 2015), but to our knowledge this is the first study aimed at exploring links between iWUE and isohydricity directly. Because iWUE is the ratio of A to  $g_s$ , an increase in iWUE of isohydric species under water limitation is possible with three potential scenarios: (1) increased A and decreased/retained/smaller increase of  $g_s$ , (2) decreased A and larger decrease of  $g_s$ , or (3) constant A and decreased  $g_s$ . Our results suggest the second scenario for the more isohydric tulip poplar and sugar maple – both  $g_s$  and A declined during water stress, but A declined more slowly – an outcome predicted from the shape of the A– $c_i$  curve absent of significant nonstomatal limitations to photosynthesis. On the other hand, static iWUE regardless of water availability, as we

have observed from white oak, is possible when the changes of A and  $g_s$  occur in the same direction with similar magnitude. In our case, white oak's A was insensitive to the variation of  $\theta$  and D (Fig. 4a,b), suggesting negligible  $g_s$  variation for white oak compared with other species, as has been confirmed in previous work performed in this site (Roman *et al.*, 2015).

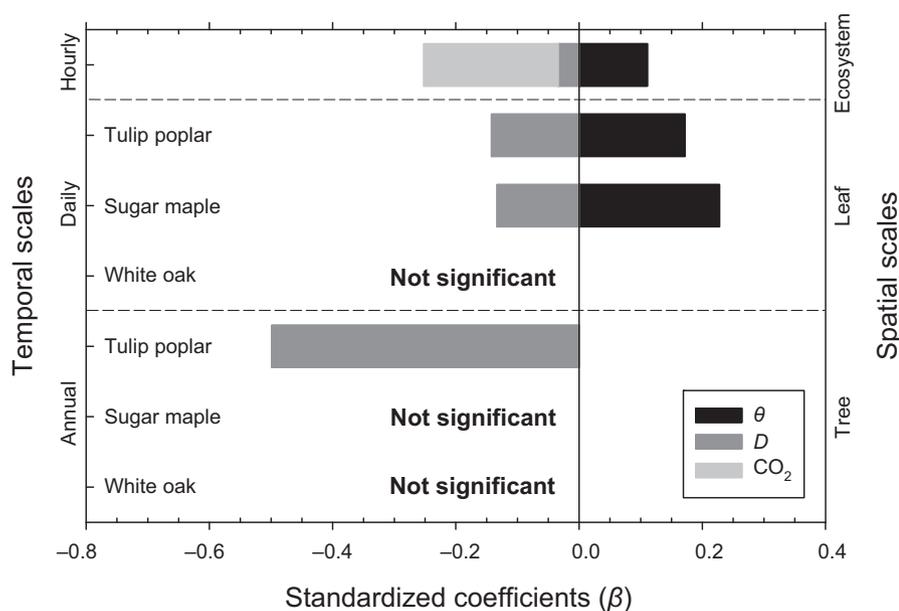
The three different approaches used here to estimate iWUE represent different observational scales and are well-documented and widely accepted methods. However, each approach is subject to its own limitations, which could cause discrepancy in iWUE estimates. The pattern of iWUE<sub>T</sub> variation was similar to that of iWUE<sub>L</sub> and iWUE\*, but its magnitude was smaller than that of iWUE<sub>L</sub> and iWUE\* in general (Fig. 2). These differences could reflect a temporal scale mismatch between the leaf and tree data.



**Fig. 5** Linear relationships between environmental drivers ((a–c) soil moisture content ( $\theta$ ), (d–f) vapor pressure deficit ( $D$ ), or (g–i) atmospheric  $\text{CO}_2$  concentration ( $c_a$ )) and annual radial growth (basal area increment, BAI) of (a, d, g) tulip poplar, (b, e, h) sugar maple, and (c, f, i) white oak. Each circle represents mean BAI of all tree-ring samples ( $n = 8$ ) in each year (1999–2012). Error bars represent plus/minus SE of the means (95% confidence). Slopes were presented only for those of significant relationships ( $P < 0.05$ ). Note that interactions between the drivers are not accounted for in this presentation; see Fig. 6 for a discussion of the relative influence of  $\theta$ ,  $D$  and  $c_a$  on growth (tree scale).

Because tree rings integrate processes occurring over an entire growing season, physiological responses to environmental signals varying at shorter timescales over which droughts occur are smoothed in the tree-ring data. Differences between leaf- and

tree-scale dynamics could also reflect the reallocation of NSCs in the tree. Trees utilize stored NSC to mediate demand from various plant functions, including growth, defense, reproduction, osmoregulation, and symbiosis, by translocating it to relevant



**Fig. 6** Standardized coefficients ( $\beta$ ) of soil moisture contents ( $\theta$ ), vapor pressure deficit ( $D$ ), and atmospheric  $\text{CO}_2$  concentration ( $c_a$ ) for the carbon (C) gain observed at the different scales in the Morgan–Monroe State Forest. The standard coefficients are the estimates resulting from a multiple regression analysis, and refer to how many SDs a C gain will change per SD increase in either the  $\theta$ ,  $D$  or  $c_a$ . The C gains are represented by gross primary production for the hourly/ecosystem scale, C assimilation rate for the daily to weekly/leaf scale, and basal area increment for the annual/tree scale. Note that the predictors without statistical significance ( $P > 0.05$ ) are not presented.

organs (Hartmann & Trumbore, 2016). NSC formation and utilization in leaves is relatively fast; for example, leaves convert sugars into nonstructural starch and store it during the day to buffer the shortage of sugar at night or to save for another usage after reallocation (Geiger *et al.*, 2000). Therefore, the temporal scale of NSC residence in the leaf is on the order of hours to days, and we can presume the C age of leaves is young, while the variation of NSC in the stem is known to occur seasonally or even throughout decades (Kozłowski & Keller, 1966). Moreover, mixing of NSC in the stem both inward (transport of young NSC) and outward (transport of old NSC) in the radial direction via vascular rays might obscure the C age across annual rings, although the NSC concentration is very small relative to structural carbohydrates. The mixing and turnover of old and new NSC might dampen the  $\delta^{13}\text{C}$  signal used as a gas-exchange proxy and thus best reflect long-term changes in NSC allocation. Therefore, we can conclude that the tree-ring approach, despite its usefulness for long-term observations, may not always be well suited for capturing tree response to fluctuating environmental conditions within a single growing season.

The  $i\text{WUE}^*$  was overall lower than  $i\text{WUE}_L$  and  $i\text{WUE}_T$ , which is expected due to the contribution of nontranspiratory water vapor flux to evapotranspiration in estimating  $i\text{WUE}$ . Previous work conducted at the site leveraging numerous approaches for partitioning ET into evaporation and transpiration concluded that, somewhat paradoxically, the relative contribution of evaporation to ET increases during drought, due to steep increases in  $D$ , which represents the driving force moving water from soil to the air (Sulman *et al.*, 2016). Thus, our estimates of the increase in  $i\text{WUE}^*$  during periods of hydrologic stress are likely conservative, as the proportion of ET that occurs as transpiration declines as drought progresses. The diversity of species growing in MMSF further challenges the interpretation of the  $i\text{WUE}^*$  dynamics. Specifically, while our three study species account for  $> 50\%$  of the leaf area in the study site (Roman *et al.*, 2015), the tower-

derived  $i\text{WUE}^*$  is likely influenced by species that were not observed at the leaf or tree scales. Undoubtedly, too, the magnitude of GPP is influenced by the choice of partitioning approach (Stoy *et al.*, 2006; van Gorsel *et al.*, 2009), and this could explain some of the discrepancy in the magnitude of  $i\text{WUE}$  across the scales. However, uncertainties related to partitioning should be smaller when comparing temporal trends in GPP and  $i\text{WUE}$ , as opposed to absolute magnitudes (Novick *et al.*, 2015).

Despite these methodological challenges, the response of  $i\text{WUE}^*$  to  $\theta$  and  $D$  was similar to the pattern of isohydric tulip poplar (Fig. 2), consistent with the fact that the tower footprint is comprised of a greater number of isohydric species than anisohydric species. In 2013, sugar maple, tulip poplar, and sassafras comprised 53% of leaf area index and were classified as isohydric species, whereas oaks (anisohydric) accounted for only 6% of leaf area index (Roman *et al.*, 2015). Therefore, this result implies that transition of species composition might significantly alter C and water cycling at the ecosystem scale, especially when the composition of trees' isohydricity changes.

Our results highlight the importance of  $D$  in driving the  $i\text{WUE}$  trends, but also demonstrate that the relative importance of environmental drivers varies depending on observational scale. At the hourly and daily scales,  $D$  influenced  $i\text{WUE}$  most, promoting variation that was two to three times higher than the variation in  $i\text{WUE}$  linked to changes in  $c_a$  (Fig. 3). Moreover, even at the annual scale, where the influence of  $c_a$  was the highest,  $D$  still showed high influence on tulip poplar, accounting for 33% of the variation in  $i\text{WUE}$ . This finding is crucial, considering much attention has focused on plant physiological response to rising  $c_a$  and/or declining  $\theta$ , but less on increasing  $D$  (Novick *et al.*, 2016). In fact, rising  $D$  has been observed not only in MMSF (Brzostek *et al.*, 2014) but also in most places across USA and is projected to continue to increase into the future (Ficklin & Novick, 2017). Therefore, failure to account for  $D$  trends will make it difficult to use historic time-series to diagnose the  $c_a$

effect on iWUE, especially when considering observations collected at different spatiotemporal scales. For example, at the annual scale, where the influence of  $D$  was relatively low (Fig. 3), tulip poplar increased iWUE with elevated  $c_a$  (Fig. 2h), which agrees with previous work (Battipaglia *et al.*, 2013; Keenan *et al.*, 2013; Frank *et al.*, 2015). However, our observations at other scales showed contrasting patterns (Fig. 2g,i), potentially due to the higher contribution of  $D$  on iWUE than  $c_a$  (Fig. 3).

In addition, our results highlight important differences in iWUE across species. At the daily scale, all species seemed to be influenced significantly by the variation of  $D$  and  $c_a$ , although the degree of the influence might differ (Fig. 3). However, tulip poplar was the only species significantly influenced by those environmental drivers in the long term. There is a major effort under way to incorporate features of plant hydraulics into terrestrial ecosystem models (Bonan *et al.*, 2014; Sperry & Love, 2015; Novick *et al.*, 2016). Ensuring that the models are formulated and parameterized to capture links between isohydricity and iWUE is critical for linking these hydraulic frameworks to predictions of C uptake. It was also noteworthy that the iWUE of the most isohydric species considered here (i.e. tulip poplar) was the most sensitive to  $\theta$  (at least at the leaf scale), whereas the other species were not (Fig. 3). These results guide several questions for future research, including whether large increases in iWUE are a generalizable trait associated with isohydricity and whether isohydricity could be an important factor explaining contradictory results using tree rings of different species to assess temporal variability in iWUE.

In conclusion, comparison of multiple spatiotemporal scales has an important implication for evaluating trees' physiological responses to environmental changes. Our comparison of the relative influence of multiple environmental drivers signified the importance of  $D$  in a tree's regulation of iWUE, which has been rising in many places and is predicted to increase nearly globally in the future (Ficklin & Novick, 2017). Therefore, care should be taken when interpreting historical time-series of iWUE, or predictions from ecological models, if they do not incorporate the influence of  $D$  on stomatal regulation explicitly. We also demonstrate that efforts to quantify the response of iWUE to varying environmental drivers depends on the observational scale, which should motivate the continuation of research into whether these scale dependencies reflect physiological phenomena or observation biases. Our results describe the physiological response of canopy-dominant trees growing in a temperate deciduous forest that typically experiences mesic conditions. Therefore, the generality of our result would benefit from the analysis of similar data in other biomes. Finally, we acknowledge the recent criticisms on the use of isohydric/anisohydric framework, pointing out the cases where tight  $\Psi_L$  regulation is not necessarily associated with greater stomatal regulation, due to a range of factors – for more details, see Martinez-Vilalta & Garcia-Forner (2017) and Hochberg *et al.* (2018). Nonetheless, the gas-exchange dynamics of our study species are explained well by the isohydric/anisohydric framework, with higher sensitivity of  $g_s$  and tighter regulation of  $\Psi_L$  for the more isohydric species (see Fig. S2 for  $\Psi_L$  and Fig. S6 for  $g_s$  in the Supporting Information),

which was further supported by pre-dawn  $\Psi_L$  observations described earlier. We recommend caution, however, when diagnosing isohydricity, and agree with the need for more thorough investigation of the factors that can potentially decouple  $g_s$  and  $\Psi_L$  regulation in future work.

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## Author contributions

K.Y., J.T.M., R.P.P., and K.A.N. planned and designed the research. K.Y., J.T.M., and D.T.R. conducted field work, and K.Y., M.K.W., and P.E.S. performed experiments. K.Y. analyzed the data and wrote the paper with input and revisions from all co-authors.

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## References

- Altieri S, Mereu S, Cherubini P, Castaldi S, Sirignano C, Lubritto C, Battipaglia G. 2015. Tree-ring carbon and oxygen isotopes indicate different water use strategies in three Mediterranean shrubs at Capo Caccia (Sardinia, Italy). *Trees: Structure and Function* 29: 1593–1603.
- Attia Z, Domec JC, Oren R, Way DA, Moshelion M. 2015. Growth and physiological responses of isohydric and anisohydric poplars to drought. *Journal of Experimental Botany* 66: 4373–4381.
- Battipaglia G, De Micco V, Brand WA, Saurer M, Aronne G, Linke P, Cherubini P. 2014. Drought impact on water use efficiency and intra-annual density fluctuations in *Erica arborea* on Elba (Italy). *Plant, Cell & Environment* 37: 382–391.
- Battipaglia G, Saurer M, Cherubini P, Calfapietra C, McCarthy HR, Norby RJ, Cotrufo MF. 2013. Elevated CO<sub>2</sub> increases tree-level intrinsic water use efficiency: insights from carbon and oxygen isotope analyses in tree rings across three forest FACE sites. *New Phytologist* 197: 544–554.
- Battipaglia G, Saurer M, Cherubini P, Siegwolf RTW, Cotrufo MF. 2009. Tree rings indicate different drought resistance of a native (*Abies alba* Mill.) and a nonnative (*Picea abies* (L.) Karst.) species co-occurring at a dry site in southern Italy. *Forest Ecology and Management* 257: 820–828.
- Beer C, Ciais P, Reichstein M, Baldocchi D, Law BE, Papale D, Soussana JF, Ammann C, Buchmann N, Frank D *et al.* 2009. Temporal and among-site

- variability of inherent water use efficiency at the ecosystem level. *Global Biogeochemical Cycles* 23: GB2018.
- Beer C, Reichstein M, Tomelleri E, Ciais P, Jung M, Carvalhais N, Rödenbeck C, Arain MA, Baldocchi D, Bonan GB *et al.* 2010. Terrestrial gross carbon dioxide uptake: global distribution and covariation with climate. *Science* 329: 834–838.
- Belmecheri S, Maxwell RS, Taylor AH, Davis KJ, Freeman KH, Munger WJ. 2014. Tree-ring  $\delta^{13}\text{C}$  tracks flux tower ecosystem productivity estimates in a NE temperate forest. *Environmental Research Letters* 9: e074011.
- Bonan GB, Williams M, Fisher RA, Oleson KW. 2014. Modeling stomatal conductance in the earth system: linking leaf water-use efficiency and water transport along the soil–plant–atmosphere continuum. *Geoscientific Model Development* 7: 2193–2222.
- Breda N, Huc R, Granier A, Dreyer E. 2006. Temperate forest trees and stands under severe drought: a review of ecophysiological responses, adaptation processes and long-term consequences. *Annals of Forest Science* 63: 625–644.
- Brienen RJW, Wanek W, Hietz P. 2011. Stable carbon isotopes in tree rings indicate improved water use efficiency and drought responses of a tropical dry forest tree species. *Trees: Structure and Function* 25: 103–113.
- Brzostek ER, Dragoni D, Schmid HP, Rahman AF, Sims D, Wayson CA, Johnson DJ, Phillips RP. 2014. Chronic water stress reduces tree growth and the carbon sink of deciduous hardwood forests. *Global Change Biology* 20: 2531–2539.
- Buckley TN. 2017. Modeling stomatal conductance. *Plant Physiology* 174: 572–582.
- Cernusak LA, Aranda J, Marshall JD, Winter K. 2007. Large variation in whole-plant water-use efficiency among tropical tree species. *New Phytologist* 173: 294–305.
- Chaves MM, Maroco JP, Pereira JS. 2003. Understanding plant responses to drought – from genes to the whole plant. *Functional Plant Biology* 30: 239–264.
- Choat B, Jansen S, Brodribb TJ, Cochard H, Delzon S, Bhaskar R, Bucci SJ, Feild TS, Gleason SM, Hacke UG *et al.* 2012. Global convergence in the vulnerability of forests to drought. *Nature* 491: 752–756.
- Dragoni D, Schmid HP, Wayson CA, Potter H, Grimmond CSB, Randolph JC. 2011. Evidence of increased net ecosystem productivity associated with a longer vegetated season in a deciduous forest in south-central Indiana, USA. *Global Change Biology* 17: 886–897.
- Farquhar GD, Ehleringer JR, Hubick KT. 1989. Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* 40: 503–537.
- Farquhar GD, Richards RA. 1984. Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. *Australian Journal of Plant Physiology* 11: 539–552.
- Farquhar GD, von Caemmerer S, Berry JA. 1980. A biochemical model of photosynthetic  $\text{CO}_2$  assimilation in leaves of  $\text{C}_3$  species. *Planta* 149: 78–90.
- Ficklin DL, Novick KA. 2017. Historic and projected changes in vapor pressure deficit suggest a continental-scale drying of the United States atmosphere. *Journal of Geophysical Research: Atmospheres* 122: 2061–2079.
- Flexas J, Barbour MM, Brendel O, Cabrera HM, Carriqui M, Diaz-Espejo A, Douthe C, Dreyer E, Ferrio JP, Gago J *et al.* 2012. Mesophyll diffusion conductance to  $\text{CO}_2$ : an unappreciated central player in photosynthesis. *Plant Science* 193–194: 70–84.
- Flexas J, Ribas-Carbo M, Diaz-Espejo A, Galmes J, Medrano H. 2008. Mesophyll conductance to  $\text{CO}_2$ : current knowledge and future prospects. *Plant, Cell & Environment* 31: 602–621.
- Frank DC, Poulter B, Saurer M, Esper J, Huntingford C, Helle G, Treydte K, Zimmermann NE, Schleser GH, Ahlstrom A *et al.* 2015. Water-use efficiency and transpiration across European forests during the Anthropocene. *Nature Climate Change* 5: 579–584.
- Geiger DR, Servaites JC, Fuchs MA. 2000. Role of starch in carbon translocation and partitioning at the plant level. *Australian Journal of Plant Physiology* 27: 571–582.
- van Gorsel E, Delpierre N, Leuning R, Black A, Munger JW, Wofsy S, Aubinet M, Feigenwinter C, Beringer J, Bonal D *et al.* 2009. Estimating nocturnal ecosystem respiration from the vertical turbulent flux and change in storage of  $\text{CO}_2$ . *Agricultural and Forest Meteorology* 149: 1919–1930.
- Grassi G, Magnani F. 2005. Stomatal, mesophyll conductance and biochemical limitations to photosynthesis as affected by drought and leaf ontogeny in ash and oak trees. *Plant, Cell & Environment* 28: 834–849.
- Guerrieri R, Lepine L, Asbjornsen H, Xiao JF, Ollinger SV. 2016. Evapotranspiration and water use efficiency in relation to climate and canopy nitrogen in US forests. *Journal of Geophysical Research: Biogeosciences* 121: 2610–2629.
- Hartmann H, Trumbore S. 2016. Understanding the roles of nonstructural carbohydrates in forest trees – from what we can measure to what we want to know. *New Phytologist* 211: 386–403.
- Hentschel R, Hommel R, Poschenrieder W, Grote R, Holst J, Biernath C, Gessler A, Priesack E. 2016. Stomatal conductance and intrinsic water use efficiency in the drought year 2003: a case study of European beech. *Trees: Structure and Function* 30: 153–174.
- Hietz P, Wanek W, Dunisch O. 2005. Long-term trends in cellulose  $\delta^{13}\text{C}$  and water-use efficiency of tropical *Cedrela* and *Swietenia* from Brazil. *Tree Physiology* 25: 745–752.
- Hochberg U, Rockwell FE, Holbrook NM, Cochard H. 2018. Iso/anisohydry: a plant–environment interaction rather than a simple hydraulic trait. *Trends in Plant Science* 23: 112–120.
- Holmes RL. 1983. Computer-assisted quality control in tree-ring dating and measurement. *Tree-Ring Bulletin* 43: 69–78.
- Keeling CD, Piper SC, Bacastow RB, Wahlen M, Whorf TP, Heimann M, Meijer HA. 2005. Atmospheric  $\text{CO}_2$  and  $^{13}\text{CO}_2$  exchange with the terrestrial biosphere and oceans from 1978 to 2000: observations and carbon cycle implications. In: Baldwin IT, Caldwell MM, Heldmaier G, Jackson RB, Lange OL, Mooney HA, Schulze ED, Sommer U, Ehleringer JR, Denise Dearing M *et al.*, eds. *A history of atmospheric  $\text{CO}_2$  and its effects on plants, animals, and ecosystems*. New York, NY, USA: Springer, 83–113.
- Keenan TF, Hollinger DY, Bohrer G, Dragoni D, Munger JW, Schmid HP, Richardson AD. 2013. Increase in forest water-use efficiency as atmospheric carbon dioxide concentrations rise. *Nature* 499: 324–328.
- Klein T, Shpringer I, Fikler B, Elbaz G, Cohen S, Yakir D. 2013. Relationships between stomatal regulation, water-use, and water-use efficiency of two coexisting key Mediterranean tree species. *Forest Ecology and Management* 302: 34–42.
- Klein T, Yakir D, Buchmann N, Grunzweig JM. 2014. Towards an advanced assessment of the hydrological vulnerability of forests to climate change-induced drought. *New Phytologist* 201: 712–716.
- Konings AG, Williams AP, Gentine P. 2017. Sensitivity of grassland productivity to aridity controlled by stomatal and xylem regulation. *Nature Geoscience* 10: 284–290.
- Kozlowski TT, Keller T. 1966. Food relations of woody plants. *Botanical Review* 32: 293–382.
- Linares JC, Camarero JJ. 2012. From pattern to process: linking intrinsic water-use efficiency to drought-induced forest decline. *Global Change Biology* 18: 1000–1015.
- Lipp J, Trimborn P, Fritz P, Moser H, Becker B, Frenzel B. 1991. Stable isotopes in tree-ring cellulose and climatic-change. *Tellus B: Chemical and Physical Meteorology* 43: 322–330.
- Livingston NJ, Spittlehouse DL. 1996. Carbon isotope fractionation in tree ring early and late wood in relation to intra-growing season water balance. *Plant, Cell & Environment* 19: 768–774.
- Loader NJ, Robertson I, Barker AC, Switsur VR, Waterhouse JS. 1997. An improved technique for the batch processing of small wholewood samples to  $\alpha$ -cellulose. *Chemical Geology* 136: 313–317.
- Mallya G, Zhao L, Song XC, Niyogi D, Govindaraju RS. 2013. 2012 Midwest drought in the United States. *Journal of Hydrologic Engineering* 18: 737–745.
- Malone SL, Tulbure MG, Perez-Luque AJ, Assal TJ, Bremer LL, Drucker DP, Hillis V, Varela S, Goulden ML. 2016. Drought resistance across California ecosystems: evaluating changes in carbon dynamics using satellite imagery. *Ecosystems* 7: e01561.
- Manzoni S, Vico G, Katul G, Palmroth S, Jackson RB, Porporato A. 2013. Hydraulic limits on maximum plant transpiration and the emergence of the safety–efficiency trade-off. *New Phytologist* 198: 169–178.
- Martínez-Vilalta J, García-Forner N. 2017. Water potential regulation, stomatal behaviour and hydraulic transport under drought:

- deconstructing the iso/anisohydric concept. *Plant, Cell & Environment* 40: 962–976.
- Martinez-Vilalta J, Poyatos R, Aguade D, Retana J, Mencuccini M. 2014. A new look at water transport regulation in plants. *New Phytologist* 204: 105–115.
- Maseyk K, Hemming D, Angert A, Leavitt SW, Yakir D. 2011. Increase in water-use efficiency and underlying processes in pine forests across a precipitation gradient in the dry Mediterranean region over the past 30 years. *Oecologia* 167: 573–585.
- McDowell N, Pockman WT, Allen CD, Breshears DD, Cobb N, Kolb T, Plaut J, Sperry J, West A, Williams DG *et al.* 2008. Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytologist* 178: 719–739.
- Medlyn BE, De Kauwe MG, Lin Y-S, Knauer J, Duursma RA, Williams CA, Arneth A, Clement R, Isaac P, Limousin J-M *et al.* 2017. How do leaf and ecosystem measures of water-use efficiency compare? *New Phytologist* 216: 758–770.
- Meinzer FC, Woodruff DR, Marias DE, Smith DD, McCulloh KA, Howard AR, Magedman AL. 2016. Mapping 'hydroscares' along the iso- to anisohydric continuum of stomatal regulation of plant water status. *Ecology Letters* 19: 1343–1352.
- Monson RK, Prater MR, Hu J, Burns SP, Sparks JP, Sparks KL, Scott-Denton LE. 2010. Tree species effects on ecosystem water-use efficiency in a high-elevation, subalpine forest. *Oecologia* 162: 491–504.
- Nepstad DC, Moutinho P, Dias MB, Davidson E, Cardinot G, Markewitz D, Figueiredo R, Vianna N, Chambers J, Ray D *et al.* 2002. The effects of partial throughfall exclusion on canopy processes, aboveground production, and biogeochemistry of an Amazon forest. *Journal of Geophysical Research: Atmospheres* 107: 8085.
- Nock CA, Baker PJ, Wanek W, Leis A, Grabner M, Bunyavejchewin S, Hietz P. 2011. Long-term increases in intrinsic water-use efficiency do not lead to increased stem growth in a tropical monsoon forest in western Thailand. *Global Change Biology* 17: 1049–1063.
- Novick KA, Ficklin DL, Stoy PC, Williams CA, Bohrer G, Oishi AC, Papuga SA, Blanken PD, Noormets A, Sulman BN *et al.* 2016. The increasing importance of atmospheric demand for ecosystem water and carbon fluxes. *Nature Climate Change* 6: 1023–1027.
- Novick KA, Oishi AC, Ward EJ, Siqueira MBS, Juang JY, Stoy PC. 2015. On the difference in the net ecosystem exchange of CO<sub>2</sub> between deciduous and evergreen forests in the southeastern United States. *Global Change Biology* 21: 827–842.
- O'Leary MH. 1981. Carbon isotope fractionation in plants. *Phytochemistry* 20: 553–567.
- Ponton S, Dupouey JL, Breda N, Dreyer E. 2002. Comparison of water-use efficiency of seedlings from two sympatric oak species: genotype × environment interactions. *Tree Physiology* 22: 413–422.
- Renninger HJ, Carlo NJ, Clark KL, Schafer KVR. 2015. Resource use and efficiency, and stomatal responses to environmental drivers of oak and pine species in an Atlantic Coastal Plain forest. *Frontiers in Plant Science* 6: e297.
- Rigden AJ, Salvucci GD. 2017. Stomatal response to humidity and CO<sub>2</sub> implicated in recent decline in US evaporation. *Global Change Biology* 23: 1140–1151.
- Roman DT, Novick KA, Brzostek ER, Dragoni D, Rahman F, Phillips RP. 2015. The role of isohydric and anisohydric species in determining ecosystem-scale response to severe drought. *Oecologia* 179: 641–654.
- Salinger M. 2005. Increasing climate variability and change: reducing the vulnerability. *Climatic Change* 70: 1–3.
- Scartazza A, Vaccari FP, Bertolini T, Di Tommasi P, Lauteri M, Miglietta F, Brugnoli E. 2014. Comparing integrated stable isotope and eddy covariance estimates of water-use efficiency on a Mediterranean successional sequence. *Oecologia* 176: 581–594.
- Schar C, Vidale PL, Luthi D, Frei C, Haberli C, Liniger MA, Appenzeller C. 2004. The role of increasing temperature variability in European summer heatwaves. *Nature* 427: 332–336.
- Schmid HP, Grimmond CSB, Cropley F, Offerle B, Su HB. 2000. Measurements of CO<sub>2</sub> and energy fluxes over a mixed hardwood forest in the mid-western United States. *Agricultural and Forest Meteorology* 103: 357–374.
- Silva LCR, Anand M, Oliveira JM, Pillar VD. 2009. Past century changes in *Araucaria angustifolia* (Bertol.) Kuntze water use efficiency and growth in forest and grassland ecosystems of southern Brazil: implications for forest expansion. *Global Change Biology* 15: 2387–2396.
- Silva LCR, Sun G, Zhu-Barker X, Liang QL, Wu N, Horwath WR. 2016. Tree growth acceleration and expansion of alpine forests: the synergistic effect of atmospheric and edaphic change. *Science Advances* 2: e1501302.
- Skelton RP, West AG, Dawson TE. 2015. Predicting plant vulnerability to drought in biodiverse regions using functional traits. *Proceedings of the National Academy of Sciences of the United States of America* 112: 5744–5749.
- van der Sleen P, Groenendijk P, Vlam M, Anten NPR, Boom A, Bongers F, Pons TL, Terburg G, Zuidema PA. 2015. No growth stimulation of tropical trees by 150 years of CO<sub>2</sub> fertilization but water-use efficiency increased. *Nature Geoscience* 8: 24–28.
- Sperry JS, Love DM. 2015. What plant hydraulics can tell us about responses to climate-change droughts. *New Phytologist* 207: 14–27.
- Stoy PC, Katul GG, Siqueira MBS, Juang JY, Novick KA, Uebelherr JM, Oren R. 2006. An evaluation of models for partitioning eddy covariance-measured net ecosystem exchange into photosynthesis and respiration. *Agricultural and Forest Meteorology* 141: 2–18.
- Sulman BN, Roman DT, Scanlon TM, Wang LX, Novick KA. 2016. Comparing methods for partitioning a decade of carbon dioxide and water vapor fluxes in a temperate forest. *Agricultural and Forest Meteorology* 226: 229–245.
- Tang XG, Li HP, Desai AR, Nagy Z, Luo JH, Kolb TE, Olliso A, Xu XB, Yao L, Kutsch W *et al.* 2014. How is water-use efficiency of terrestrial ecosystems distributed and changing on Earth? *Scientific Reports* 4: e7483.
- Tardieu F, Simonneau T. 1998. Variability among species of stomatal control under fluctuating soil water status and evaporative demand: modelling isohydric and anisohydric behaviours. *Journal of Experimental Botany* 49: 419–432.
- Tezara W, Mitchell VJ, Driscoll SD, Lawlor DW. 1999. Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. *Nature* 401: 914–917.
- Tyree MT, Sperry JS. 1988. Do woody plants operate near the point of catastrophic xylem dysfunction caused by dynamic water stress?: answers from a model. *Plant Physiology* 88: 574–580.
- Vickers D, Thomas CK, Pettijohn C, Martin JG, Law BE. 2012. Five years of carbon fluxes and inherent water-use efficiency at two semi-arid pine forests with different disturbance histories. *Tellus B: Chemical and Physical Meteorology* 64: 17159.
- Williams AP, Allen CD, Macalady AK, Griffin D, Woodhouse CA, Meko DM, Swetnam TW, Rauscher SA, Seager R, Grissino-Mayer HD *et al.* 2013. Temperature as a potent driver of regional forest drought stress and tree mortality. *Nature Climate Change* 3: 292–297.
- Yi K, Dragoni D, Phillips RP, Roman DT, Novick KA. 2017. Dynamics of stem water uptake among isohydric and anisohydric species experiencing a severe drought. *Tree Physiology* 37: 1379–1392.

## Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

**Fig. S1** Annual NEE and ET from 1999 to 2013.

**Fig. S2** Relationship between  $\Psi_S$  and mid-day  $\Psi_L$ .

**Fig. S3** Mean daily variation of  $\theta$ ,  $D$  and  $c_a$  from 1999 to 2013.

**Fig. S4** Annual BAI for tulip poplar, sugar maple and white oak.

**Fig. S5** Mean  $\theta$ ,  $D$  and  $c_a$  during the late growing season (June–August) from 1999 to 2013.

**Fig. S6** Variation of  $g_s$  at the leaf-scale and  $g_c$  at the ecosystem-scale.

**Fig. S7** Variation of  $E$  at the leaf-scale and ET at the ecosystem-scale.

**Table S1** Mean pre-dawn  $\Psi_L$ .

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