Original Article

Drivers of radial growth and carbon isotope discrimination of bur oak (*Quercus macrocarpa* Michx.) across continental gradients in precipitation, vapour pressure deficit and irradiance

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ABSTRACT

Tree-ring characteristics are commonly used to reconstruct climate variables, but divergence from the assumption of a single biophysical control may reduce the accuracy of these reconstructions. Here, we present data from bur oaks (*Quercus macrocarpa* Michx.) sampled within and beyond the current species bioclimatic envelope to identify the primary environmental controls on ring-width indices (RWIs) and carbon stable isotope discrimination ($\Delta^{13}C$) in tree-ring cellulose. Variation in $\Delta^{13}C$ and RWI was more strongly related to leaf-to-air vapour pressure deficit (VPD) at the centre and western edge of the range compared with the northern and wettest regions. Among regions, $\Delta^{13}C$ of tree-ring cellulose was closely predicted by VPD and light responses of canopy-level $\Delta^{13}C$ estimated using a model driven by eddy flux and meteorological measurements ($R^2 = 0.96$, $P = 0.003$). RWI and $\Delta^{13}C$ were positively correlated in the drier regions, while they were negatively correlated in the wettest region. The strength and direction of the correlations scaled with regional VPD or the ratio of precipitation to evapotranspiration. Therefore, the correlation strength between RWI and $\Delta^{13}C$ may be used to infer past wetness or aridity from paleo wood by determining the degree to which carbon gain and growth have been more limited by moisture or light.

Key-words: CO_2; dendrochronology; drought; growth; humidity; light; stable isotopes; stomata.

INTRODUCTION

Dendrochronology has become an invaluable tool for deciphering modes of past climate variability that may not be apparent from contemporary meteorological records. Two principles underlying dendrochronological climate reconstructions are that (1) tree-ring patterns are governed by the most limiting environmental conditions to plant growth processes and (2) the most limiting factor determining recent variation in tree rings has operated uniformly in the past. By appropriate sampling of species and sites, these conditions have often been met and thus tree-ring-based climate reconstructions may be valid over long timescales (Speer 2010). However, multiproxy methods need to be developed for conditions in which the principle of uniformity is invalid. It is well known that at most sites multiple resources often interact during a growing season such that no single resource is distinguishable as limiting the annual growth of a plant (Bloom, Chapin III & Mooney 1985; Chapin *et al*. 1987). Moreover, the dominant resource limitation may differ over time if the magnitude of climate change interacts with nonlinear or threshold-type biological or ecological responses. For instance, in many regions across North America, the last deglaciation was characterized by long-term warming punctuated by rapid climate shifts as well as increased seasonality in insolation and temperatures (Williams & Jackson 2007). During this period, oaks from central North America showed long-term shifts in radial growth and wood anatomy, which may be indicative of switches of the primary environmental limitations to photosynthetic carbon gain and/or cambial activity (Guyette *et al*. 2006; Voelker *et al*. 2012). Likewise, increasing atmospheric [CO_2] and global warming predicted for the coming centuries (IPCC 2007) are certain to diminish the uniformity of responses of tree growth to climate, which already appears to be occurring in some regions (Knapp, Soulé & Grissino-Mayer 2001; D'Arrigo *et al*. 2008; Way & Oren 2010; Wyckoff & Bowers 2010; Lloyd, Bunn & Berner 2011). Stable carbon isotope ratios in tree rings have long been touted as promising signals for use in paleoclimatology (Wilson & Grinstead 1977; Mazany, Lerman & Long 1980; Tans & Mook 1980), particularly when these signals are interpreted with mechanistic models (Francey & Farquhar 1982; McCarroll & Loader 2004). Because stable isotope ratios improve our ability to partition variation among the dominant environmental factors influencing growth, they should provide clearer insight into when ring-width-based climate

reconstructions diverge from uniformity of a primary environmental driver.

In North America, bur oak (*Quercus macrocarpa* Michx.) occurs across continental gradients in precipitation, leaf internal to atmospheric vapour pressure deficit (VPD) and irradiance; its range extends across the northern edge of the eastern deciduous forest, across much of the Great Plains and south to the Gulf of Mexico (Burns & Honkala 1990). Bur oak occurs in upland forests, particularly forests where disturbances such as fire have been frequent in the past (Grimm 1984), but is most widespread in riparian zones where flooding disturbances are common. In Missouri (MO), USA, near the centre of bur oak's range, ring widths from extant trees and sub-fossil oak wood from riparian forests have been used to reconstruct a millennial-length record of drought or wetness (Stambaugh *et al*. 2011). Another study characterized wood anatomical features of riparian bur oaks across much of the bioclimatic envelope of this species. This variation was correlated with spring temperatures and was used to create a modern proxy for spring temperatures, which was then applied to sub-fossil wood with radiocarbon dates from the last deglaciation (Voelker *et al*. 2012). For the current study, we compare the responses to meteorological variables of leaf gas exchange data as well as ring widths and carbon stable isotope signals from bur oaks in the same study regions of MO, Wisconsin (WI) and South Dakota (SD). To expand the range of climatic conditions influencing the carbon stable isotopic signals of bur oak wood, we also collected branch wood from irrigated bur oaks planted outside of the normal range of the species in semi-arid (Salt Lake City, UT) and Mediterranean (Davis, CA) climates.

Combined with dendrochronological or radiocarbon dating techniques, variation in carbon isotope discrimination ($\Delta^{13}C$) recorded in cellulose has great potential for elucidating past tree physiological responses and for helping reconstruct past climatic conditions (McCarroll & Loader 2004; Ward *et al*. 2005; Gerhart *et al*. 2012). During C_3 photosynthesis, $\Delta^{13}C$ is related directly to the ratio of $[CO_2]$ within the chloroplasts (c_c) to that in the surrounding atmosphere (c_a). In the strict sense, a single environmental factor cannot completely control tree-ring $\Delta^{13}C$ because instantaneous $\Delta^{13}C$ values in the leaves are governed by photosynthetic rates as well as the stomatal and mesophyll conductances (Farquhar 1983; Evans *et al*. 1986; Farquhar, Ehleringer & Hubick 1989). However, despite the complex controls on carbon isotope discrimination, a single environmental factor can dominate the signal, thus providing a strong empirical tool for climate reconstruction. In many cases, $\Delta^{13}C$ is modulated primarily by stomatal conductance, through responses to VPD (Comstock & Ehleringer 1992; Panek & Waring 1997) and soil moisture (Saurer, Allen & Siegwolf 1997; Leavitt *et al*. 2007; Roden & Ehleringer 2007). Under low water stress, stomatal conductance decreases quickly and predictably with increasing VPD with an offset related to the negative xylem pressure regime under which the leaves developed (Woodruff, Meinzer & McCulloh 2010). Besides direct leaf-level responses of stomata to VPD, stomatal conductance of ring-porous trees may be reduced as a result of loss of a substantial portion of stem hydraulic conductance on a diurnal basis (Litvak, McCarthy & Pataki 2012). By contrast, soil water deficits act over longer timescales to induce stomatal closure (Will *et al*. 2013).

One factor that potentially weakens the link between $\Delta^{13}C$ and stomatal responses to VPD and soil moisture is variation in mesophyll conductance (Seibt *et al*. 2008). Mesophyll conductance (g_m) modulates $\Delta^{13}C$ through its effects on the $[CO_2]$ gradient between the sub-stomatal chamber and the chloroplast (c_i-c_c). For C_3 tree species, g_m tends to be greater in deciduous angiosperms than evergreen angiosperms or conifers (Flexas *et al*. 2012). For deciduous oak species, g_m accounts for about one-third of the total drawdown between c_a and c_c (Manter & Kerrigan 2004; Niinemets *et al*. 2009). Water stress has consistently been shown to decrease g_m, but increased VPD in the absence of soil water deficits appears to have no effect (Warren 2008 *and references therein*). Reports of responses of g_m to light and c_a are mixed, which may be a function of species differences or interactions with other variables (Flexas *et al*. 2007, 2008; Tazoe *et al*. 2009; Schäufele, Santrucek & Schnyder 2011). Mesophyll conductance increases at higher temperatures, but the associated changes in carbon isotope discrimination are offset by photorespiration (Evans & von Caemmerer 2013). At the leaf scale, different means of estimating g_m using the full version of the Farquhar model for carbon isotope discrimination (*sensu* Farquhar, O'Leary & Berry 1982) did not help explain diurnal and seasonal patterns better than a simplified version (Bickford, Hanson & McDowell 2010). Meanwhile, at the canopy scale, g_m was shown to vary little for three tree species tracked across growing season conditions in northern Idaho, USA (Ubierna & Marshall 2011). Effects on $\Delta^{13}C$ through modified g_m have been clearly shown for soil water deficits (Warren 2008) or those water potential gradients associated with tree height (Woodruff *et al*. 2009). However, because the riparian trees we investigated should all have had consistent access to deep soil water, we expected that variation in tree-ring $\Delta^{13}C$ would largely be influenced by stomatal responses to VPD.

Plant tissue $\Delta^{13}C$ has been shown to respond to experimental shifts in irradiance (Smith, Oliver & McMillan 1976) across a canopy openness gradient (Ehleringer *et al*. 1986; Jackson *et al*. 1993; Leavitt 1993) and to year-to-year variation in irradiance at the northern edge of a species' range (McCarroll *et al*. 2003; Young *et al*. 2010, 2012). Greater VPD or irradiance are reflected by reduced $\Delta^{13}C$, potentially making the relative influences of these factors difficult to differentiate if they co-vary. VPD strongly affects stomatal conductance and thus assimilation rates to a slightly lesser extent, whereas irradiance levels have a comparatively greater effect on assimilation rate than stomatal conductance. Greater irradiance should be accompanied by greater growth, whereas greater VPD should be accompanied by lower growth. As such, for trees growing on rich alluvial soils with consistent soil water availability, variation in $\Delta^{13}C$ should be influenced most greatly by stomatal conductance (in response to VPD) and photosynthetic rate (in response to irradiance). Indeed, oak forest canopy $\Delta^{13}C$ has shown these responses to diurnal and

seasonal changes in VPD and irradiance (Baldocchi & Bowling 2003) and growth rates could be used to resolve which environmental variable was more influential.

The goal of this study was to use stem wood of extant riparian bur oaks to evaluate the potential for reconstructing meteorological signals relevant for studies of recent climate change effects or for interpreting 'no-analogue' climates of the past (e.g. Williams & Jackson 2007) using sub-fossil wood or leaves. To accomplish this goal, we sampled individual tree rings and branch wood of bur oaks growing under wide-ranging meteorological conditions to test whether (1) there are differences in the dominant controls over inter-annual growth of bur oak trees across its range; (2) gradients in VPD and irradiance are the primary controls over tree-ring $\Delta^{13}C$; and (3) the strength and direction of correlations between ring-width indices and $\Delta^{13}C$ change across the range of bur oak.

MATERIALS AND METHODS

Sampling locations

Bur oak trees were sampled in 2009 from the south-central portion of their range in MO, the northeastern range edge in WI and the western range edge in SD (Table 1). Growing seasons are humid in MO and WI, but MO has a warmer and longer growing season, more irradiance and greater precipitation. In SD, the length of the growing season is similar to WI but there is greater irradiance, warmer temperatures and drier air (Table 1). To expand the range of environmental conditions in which trees were sampled, we also collected branch segments from 7 and 10 bur oak trees growing on the campus of the University of Utah (UT) and the campus of the University of California at Davis (CA), respectively.

In MO and WI, the trees sampled had largely regenerated after forest cutting between 50 and 100 years previously, or were scattered older trees (i.e. 150+ years in age) that were not cut during this time. These stands had likely been grazed by cattle in the past, but there was no recent evidence of logging or grazing. In SD, there was little evidence of any historic

logging and no evidence of recent logging. In SD, grazing by bison occurs but there was no evidence of intense grazing or soil disturbance near the trees sampled. In MO and WI, the stands sampled had a closed canopy but the trees sampled were generally located along forest edges associated with the riparian zones. In SD, about half of the stands sampled had a closed canopy and half were more savannah-like with a grass understory. Despite these differences in stand structure, the effects of competition should be minimized because we only selected outwardly healthy trees in a dominant or co-dominant canopy position. Heights were not recorded for each tree but the average height of the dominant trees was recorded at each stand as part of the field notes. The trees in WI and MO had similar heights of 20–24 m for trees near 90 years or older, with trees growing on forest edges tending to be shorter. Heights of SD oaks of the same age tended to be 18–20 m in closed canopy stands but only 16–18 m in open grown stands. Oaks located on upland soils in SD were much shorter and even shrub-like on harsher sites.

Within each region, trees were sampled across numerous mapped soil units according to soil survey data published for each county by the United States Department of Agriculture. These trees were always located within or adjacent to riparian areas so the soils were generally alluvial or colluvial. At each location stones, cobbles or gravel tended to be more abundant at the soil surface nearer to the stream or river channels. In MO, the upland soils surrounding the sampling areas were largely constituted of wind-blown loess that overlays limestone parent material. In WI, upland soils surrounding the sampling areas derived from either loamy or clayey till. In SD, upland soils surrounding the sampling areas were derived from metamorphic rock and granite. In each region, there was evidence of past flooding but there was no intensive farming located upstream that would have deposited sediment from tilled and/or fertilized fields.

Increment core and wood collection

In MO, stands were sampled in the vicinity of the Baskett Wildlife Area. In WI, about half of the increment cores were

Table 1. Selected characteristics from each study region and output from the program COFECHA that is used to check cross-dating and characterize ring-width data. A dash indicates no data are available. Meteorological variables are growing season means. Error values in parentheses are equal to one standard deviation calculated among years

Study area	Missouri	South Dakota	Wisconsin	California	Utah
Latitude & Longitude	38.8°N, 92.3°W	43.7°N, 103.5°W	46.7°N, 91.6°W	38.5°N, 121.8°W	40.8°N, 111.8°W
Elevation (m)	190	1300	210	15	1500
Temperature (°C)	22.7 (1.0)	17.8 (1.1)	15.5 (1.0)	21.5 (0.9)	21.1 (1.0)
Precipitation (mm d^{-1})	3.3 (0.9)	2.0 (0.6)	2.9 (0.7)	0.2 (0.1)	1.1 (0.4)
VPD (kPa)	1.32 (0.24)	1.59 (0.26)	0.93 (0.14)	2.46 (0.20)	2.64 (0.26)
PFD ($\mu mol\ m^{-2}\ s^{-1}$)	895 (59)	721 (6)	618 (58)	1170 (25)	1048 (115)
Sampled trees	33	29	47	10	7
Oldest tree (years)	185	400	314	–	–
Mean series length (years)	93	179	149	–	–
Mean sensitivity	0.249	0.210	0.208	–	–
Series inter-correlation	0.597	0.484	0.525	–	–

PFD, photon flux density; VPD, vapour pressure deficit.

collected from Brule River State Forest next to perennial streams above the Brule River. The other increment cores were sampled from oaks growing adjacent to the St. Croix River, to the east of Crex Meadows State Wildlife Area. In SD, bur oaks were sampled from four drainages in the eastern half of Custer State Park. An increment borer was used to extract two 5.15-mm-diameter cores at breast height from perpendicular radii in each tree. In UT and CA, the collection of increment cores was not permitted, so a pole pruner was used to excise three sunlit branches (2–4 cm in diameter) from each tree (Table 1). Tree rings in the branches revealed a range of 4–12 years of growth. For isotopic analyses, the wood from the branches was pooled for each tree.

Increment core cross-dating and preparation for isotope analyses

Increment cores were mounted on wooden staves and surfaced with progressively finer sandpaper. High pressure compressed air was used to clean sanding dust from the cores that would later be analysed for stable isotopes. A stereo microscope interfaced with a conventional measurement system (Velmex, Inc., Bloomfield, NY, USA) was used to measure the ring widths to 0.01 mm precision. Tree-ring series were initially cross-dated visually using marker years. The computer program COFECHA (Cook & Holmes 1986) was then used to control the dating of the series statistically (Grissino-Mayer 2001). One core from selected trees was destructively sampled for isotope analyses after cross-dating.

Bur oaks have ring-porous wood anatomy requiring earlywood vessel formation in spring before leaves have started expanding (Voelker *et al.* 2012). The timing of earlywood formation necessitates the exclusive use of stored non-structural carbohydrates, thereby imparting earlywood with an enriched Δ¹³C signal compared to latewood (Helle & Schleser 2004). However, the strong correlation between latewood Δ¹³C of a given year and earlywood Δ¹³C of the following year (Robertson *et al.* 1997) demonstrates that the same environmental signals can be preserved. Therefore, we combined the latewood for a given calendar year with the earlywood formed during the following year. Tree ring widths <1.0 mm were not used for isotope analyses because the large rays and uneven ring boundaries made accurate excision nearly impossible.

For stable isotope measurements, wood corresponding to years in the upper or lower 10 percentiles for high or low growing season mean relative humidity values was excised from cores with a razor blade. This resulted in tree rings from at least six hot and dry years and six cool and wet years being sampled for each region (Fig. 1). The rings from each nominal year were pooled into a single sample and ground to a powder. Wood powder was heat-sealed within a polyester filter bag (mesh size 25 μm; ANKOM Technology, Macedon, NY, USA), extracted to α-cellulose (Leavitt & Danzer 1993) and purified to cellulose nitrate (Sternberg 1989). Analyses of tree rings from individual trees was not possible because cellulose nitrate (for forthcoming analyses of δD) requires a
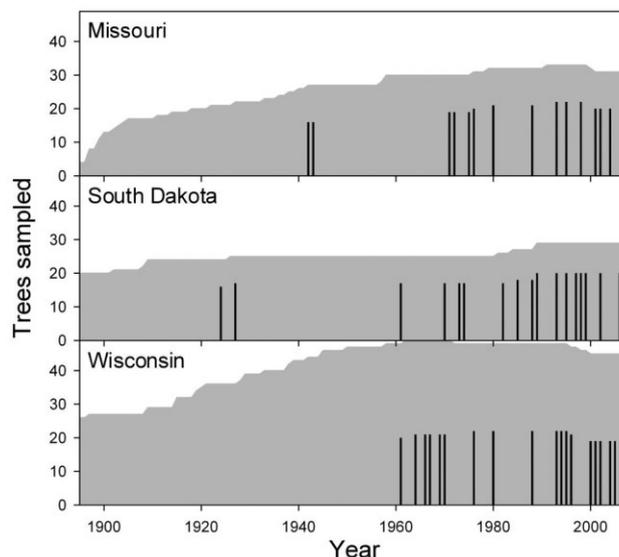

Figure 1. Number of trees sampled for each of three collection regions for ring widths (grey area) and the selected years sampled for δ¹³C (histogram).

much greater sample mass of α-cellulose than these single tree rings can yield (Sternberg 1989).

Ring-width detrending and standardization

Each ring-width series was detrended and standardized using the program ARSTAN (Cook & Holmes 1986) to produce ring-width index (RWI) chronologies. All cross-dated tree-ring measurements were used, regardless of apparent climate sensitivity, to establish the climatic signal contained in the average dominant or co-dominant trees. The ARSTAN chronologies used a conservative detrending approach, with each ring-width series being detrended twice with either of a linear or negative exponential regression in the first round and a linear regression in the second round. The chronologies were calculated using variance stabilization to minimize changes in climate sensitivity with mean tree age within each chronology.

After detrending, the mean RWI value of each chronology is equal to one with inter-annual variation being preserved, while differences in growth owing to age-related trends, tree age distributions and regional productivity were removed. To compare the RWI responses to meteorological variables across regions, we restored variation in regional productivity. Because each chronology had a different tree age distribution, it was necessary to re-scale each ARSTAN RWI chronology rather than re-scaling at the tree level. We also added in a term to account for differences in the potential photosynthetic carbon gain owing to differences in growing season irradiance. These regional adjusted ring-width indices (RWI$_a$) were calculated as follows:

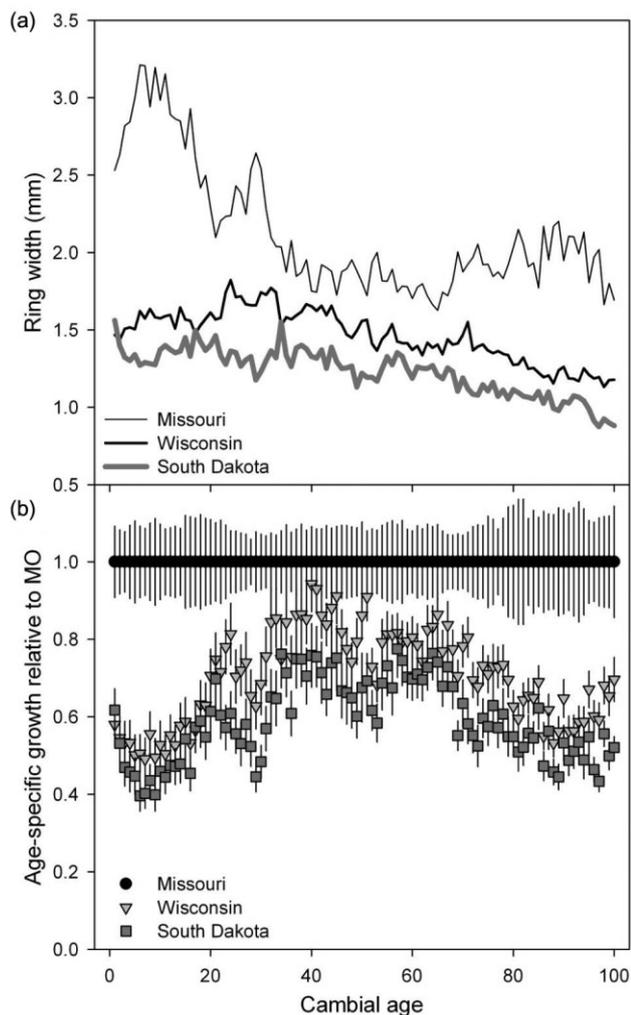$$RWI_a = \frac{RWI \times ASG_r}{A_r \times T_r},$$

Figure 2. Mean ring widths for each region plotted by cambial age (a). Patterns in mean age-specific growth (ASG$_r$), see the *Materials and Method* sections) plotted by cambial age (b). Error bars are (±1 SE among trees).

where ASG$_r$ is the age-specific growth at each region divided by the corresponding value of MO trees, A_r is the assimilation rate for a given level of regional growing season irradiance after Hamerlynck & Knapp (1996) divided by the MO value and T_r is the total growing season hours of daylight (minimum photosynthetic photon flux density, PFD = 50 μmol m^{-2} s^{-1}) divided by the MO value. To calculate ASG$_r$ among regions, ring widths were aligned by cambial age and the values for each ring divided by the mean value at a given age for the MO trees (Fig. 2). This resulted in the average age-specific growth of trees in MO equal to one and age-specific growth of trees in WI and SD being less (0.703 and 0.589, respectively) because of their slower growth during the first 100 years (Fig. 2). The irradiance used to solve for net photosynthetic rates was equal to the product of the regional growing season average PFD (see below) and the fraction of absorbed light for a hardwood canopy of 0.845 (Waring *et al.* 1995). For WI and SD, A_r was equal to 0.945 and 0.965 and T_r was equal to 0.690 and 0.707, respectively. As a result of this

computation, the RWI and RWI$_a$ from MO are equivalent because ASG$_r$, A_r and T_r all have values of one.

Climate and environmental data

Monthly climate estimates for the years 1895–2008 were obtained for each region from the PRISM climate group (http://www.prism.oregonstate.edu/). Growing season means were computed for each climate variable by weighting the monthly values by the growing degree days (baseline of 5 °C) in that month. Atmospheric and leaf internal vapour pressures were calculated from the algorithm for saturated vapour pressure reported by Paw & Gao (1988) using air temperature and dew point temperatures. For each region, growing season average evapotranspiration (ET) estimates were obtained from a dataset that used 8 day means of meteorological and MODIS remotely sensed data for the years 2000–2006 at the scale of 0.05° (see Mu *et al.* 2007, 2009 for details regarding the calculation of ET). These data were scaled up to a gridded 0.5° regional average for use in this study. PFD measurements were obtained from Ameriflux sites (http://public.ornl.gov/ameriflux/) for each region except Salt Lake City, UT, where PFD data came from meteorological stations (years 1983–1991) in the Red Butte Canyon Natural Research Area (http://ecophys.utah.edu/download/Red_Butte_Weather/). PFD data were obtained from the following Ameriflux sites and years: Missouri Ozark (MO, 2004–2009), Park Falls (WI, 2006–2009), Black Hills (SD, 2004–2008), Tonzi Ranch (CA, 2001–2010).

Isotope analyses

Subsamples (1–2 mg) of cellulose nitrate were weighed, sealed in tin capsules and sent to the Stable Isotope Facility at the University of California at Davis for isotope analyses. Samples were combusted at 1000 °C using a PDZ Europa ANCA-GSL elemental analyser interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd, Cheshire, UK). All δ^{13}C values are expressed relative to the PDB standard in‰:

$$\delta^{13}C = \left(\frac{R_{sample}}{R_{standard}} - 1\right)1000, \qquad (1)$$

where R is the ratio of ^{13}C to ^{12}C atoms of the sample or standard. The standard deviation of nine δ^{13}C replicates of our working standard α-cellulose was 0.034‰. We independently confirmed the δ^{13}C values from UC Davis were accurate by re-analysing a subset of the same cellulose nitrate values at the Stable Isotope Laboratory at Southern Oregon University where there was strong agreement between the laboratories ($R^2 = 0.99$). The standard deviation of δ^{13}C replicates of cellulose nitrate was 0.059‰.

Carbon isotope discrimination

To account for changes in c_a and $\delta^{13}C_{air}$ over time that affect the δ^{13}C of tree rings, we obtained annual values of

atmospheric [CO_2] and $\delta^{13}C_{air}$ (McCarroll & Loader 2004). For the years thereafter, the former data compilation was joined with the records from Mauna Loa, HI (http://cdiac .ornl.gov/). From this combined dataset, we converted tree-ring carbon isotope ratios ($\delta^{13}C_{plant}$) to carbon isotope discrimination values, $\Delta^{13}C$, following Farquhar (1983):

$$\Delta^{13}C = \frac{\delta^{13}C_{air} - \delta^{13}C_{plant}}{1 + \delta^{13}C_{plant}/1000}. \qquad (2)$$

We compared our tree-ring $\Delta^{13}C$ values to canopy-integrated $\Delta^{13}C$ estimates with the *CANISOTOPE* model parameterized for an oak-dominated forest and driven with hourly meteorological and eddy-flux measurements, and tested against gradients in canopy-air $\delta^{13}C$ and [CO_2] and leaf $\delta^{13}C$ from the same forest (Baldocchi & Bowling 2003). For the forest used to parameterize the model, at Walker Branch, TN, the oak species differed (i.e. *Quercus alba* L. and *Quercus prinus* L.) but these species are both closely related to bur oak and have overlapping species distribution ranges, suggesting the leaf gas exchange responses to VPD and PFD should be comparable to that of a bur oak-dominated canopy. The canopy-integrated $\Delta^{13}C$ responses to VPD and PFD were established by fitting linear regressions to the hourly data provided to us by D. Baldocchi. Because leaf $\Delta^{13}C$ tends to decrease with height in a tree or forest canopy (Ehleringer *et al.* 1986; Brooks *et al.* 1997; Duursma & Marshall 2006), canopy-integrated $\Delta^{13}C$ will tend to be greater than that of the dominant and co-dominant trees whose leaves are largely displayed high in the canopy. Wood $\Delta^{13}C$ also tends to be lower than leaf $\Delta^{13}C$ (Cernusak *et al.* 2009b). To address these issues, we used the slopes from canopy-integrated $\Delta^{13}C$ responses to VPD and light estimated by Baldocchi & Bowling (2003) but modified the intercept values to minimize the sums of the squared errors when fitted to all tree-ring $\Delta^{13}C$ measurements.

Leaf gas exchange

In SD, 7–12 fully expanded leaves of four bur oak trees were sampled for leaf gas exchange responses to VPD using a Li-6400 portable gas exchange system (Li-Cor, Inc., Lincoln, NE, USA) during morning to mid-day on 12–17 July 2009. We sampled sun leaves displayed on low branches that were exposed to full mid-day sun. The trees were centrally located among the tree-ring sampling sites in an open meadow next to a perennial stream just above Center Lake. Photosynthetic rates were measured at a PFD of 1500 μmol m^{-2} s^{-1}. The mean leaf temperature during the VPD response curve measurements was 23.3 °C (SD = 2.53). Separate temperature response curves indicated this was near the optimal temperature for this species, suggesting variation in temperatures should not have biased our VPD responses. Photosynthetic rates and stomatal conductance were similar to previous studies of gas exchange in bur oaks (Knapp 1992; Hamerlynck & Knapp 1996) so the VPD response curves were not replicated elsewhere. Moreover, there is little

evidence of acclimation of photosynthetic metabolism across latitudes and growing season conditions (Dillaway & Kruger 2011, 2012).

Statistical analyses

We used ordinary least squares techniques to test for a common slope among regions of $\Delta^{13}C$ versus VPD and to test whether the slopes from any of the three regions were different than the slope predicted at the canopy level by Baldocchi & Bowling (2003). For these tests, we used standardized major axis tests and routines (SMATR) program version 2.0 (Warton *et al.* 2006). Where slopes were not different, the same software was used to conduct post-hoc Wald tests for shifts along the *X*-axis in residuals from the common slope or for shifts along the common slope among regions. Two-tailed *t*-tests were used to compare ASG_r among MO, SD and WI with each tree serving as a replicate.

RESULTS

Comparisons of controls on tree-ring growth and gas exchange across regions

Oaks from MO had the largest ring widths across cambial ages 1–100, whereas growth of SD oaks was least and WI oak growth was intermediate (Fig. 2a). Indeed, ASG_r (Fig. 2b) of bur oaks in WI and SD were significantly lower than in MO (*t*-tests, $P < 0.016$). RWIs from MO and SD both showed strong significant negative correlations with growing season VPD (Fig. 3a). For WI bur oaks, correlations with growing season VPD were substantially lower and barely reached significance (Fig. 3a). RWIs from the SD bur oaks were significantly correlated with the previous growing season VPD ($r = -0.42$), but not for oaks in MO or WI (*data not shown*). Compared with VPD, precipitation generally showed less consistent monthly correlations with RWI chronologies (Fig. 3b). However, the growing season averages indicated that MO oaks had higher correlations with precipitation than those of SD, while for WI oaks, this correlation was lowest and not significant.

Across regions, RWI_a was negatively correlated with growing season VPD (Fig. 4a; $r = -0.57$, $P < 0.001$). This combined response was greater than the correlations from individual regions (Fig. 3a). The correlation of RWI_a and growing season precipitation was also significant (Fig. 4b; $r = 0.43$, $P < 0.001$) and greater than the correlations for WI and SD, but not that from MO (Fig. 3b) largely because the range of growing season precipitation was larger in MO than for the other regions.

Influence of VPD on leaf gas exchange

Over the range of growing season VPD values observed across regions (Fig. 4a; 0.6–2.5 kPa), there was more than a threefold difference in leaf-level stomatal conductance (Fig. 5a; $R^2 = 0.87$, $P < 0.001$) and nearly a twofold difference in leaf-level photosynthetic rates (Fig. 5b; $R^2 = 0.69$,
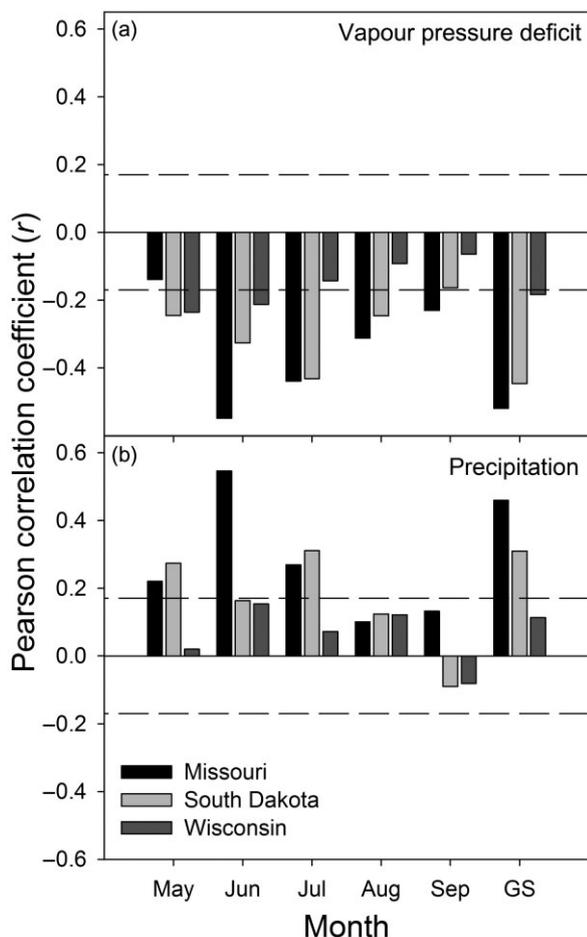
Figure 3. Correlations between ring-width indices (RWIs) and vapour pressure deficit (VPD) (a). Correlations between RWI and precipitation (b). The growing season average values are denoted as GS. The dashed lines indicate approximately where the 95% significance level occurs for a Gaussian white noise process ($n = 113$).

$P < 0.001$). Although these data cannot be simply scaled to the canopy level, they clearly indicate that high VPD values reduce stomatal conductance in bur oak and thus constrain tree carbon gain.

Influence of VPD and irradiance on $\Delta^{13}C$

Data provided by D. Baldocchi [i.e. Baldocchi & Bowling (2003)] allowed us to estimate the VPD response of canopy-integrated $\Delta^{13}C$ for an oak dominated forest as $\Delta^{13}C = -1.3910 \times VPD + 23.54$ (Fig. 6a; $R^2 = 0.78$, $P < 0.001$). Similarly, the linear light–response relationship was $\Delta^{13}C = -0.0014 \times PFD + 23.64$ (Fig. 6b; $R^2 = 0.52$, $P < 0.001$). A negative exponential relationship could also reasonably be fitted to these data as $\Delta^{13}C = 21.0253 + 3.2129 \times e^{-0.0012 \times PFD}$ (not shown, $R^2 = 0.56$, $P < 0.001$). Tree-ring $\Delta^{13}C$ for MO, WI and SD oaks each had negative relationships with growing season VPD (Fig. 7). Although we show regressions fitted for each region, these relationships were not significantly different

from each other (common slope = -0.497, $P = 0.148$ for slope differences). The slope across all data points (including tree-level variation at CA and UT) was -1.17, while that across regional means of $\Delta^{13}C$ and VPD was -1.57, indicative of shallower slopes characterizing year-to-year variation within a region compared with that observed across regions. Post-hoc Wald tests indicated that after accounting for variation in VPD (i.e. the common slope of -0.497), residuals from each of MO, SD and WI were not significantly different ($P = 0.088$), indicating that influences outside of VPD did not strongly affect $\Delta^{13}C$ among these three regions. Along the common slope, Wald tests indicated that the $\Delta^{13}C$ values of oaks from each region were significantly shifted with respect to each other ($P < 0.021$), suggesting that the differences in $\Delta^{13}C$ among regions largely owed to differences in growing season VPD.
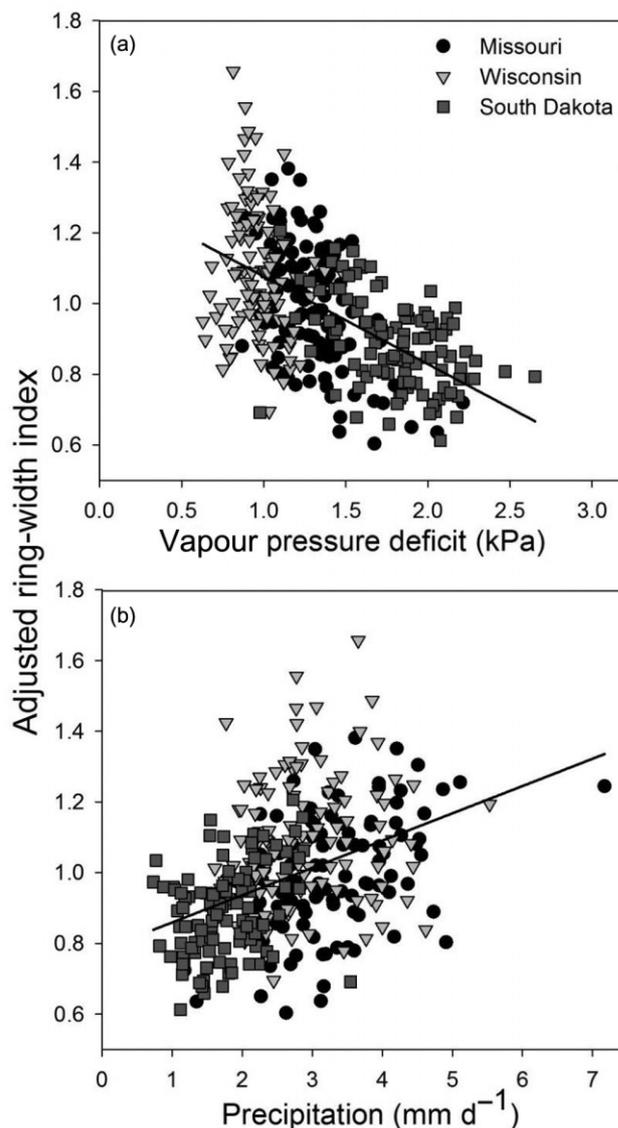

Figure 4. Adjusted ring-width index (RWI$_a$) plotted versus growing season vapour pressure deficit (VPD) (a) and precipitation (b).
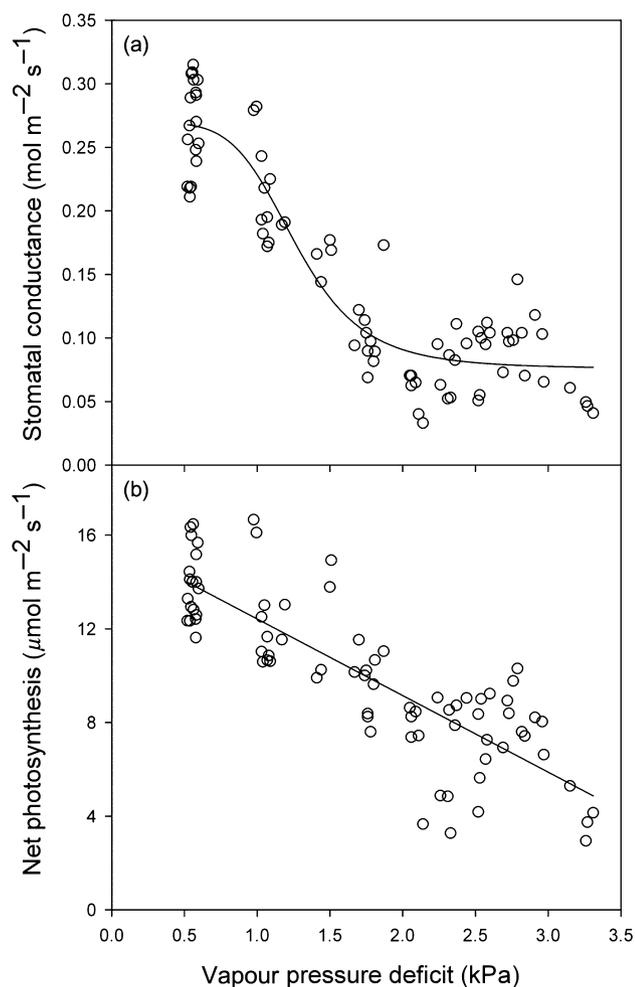
Figure 5. Gas exchange measurements made on leaves of bur oaks show vapour pressure deficit responses of stomatal conductance (a) and net photosynthesis (b). The least squares regression line fitted to stomatal conductance values was a four-parameter logistic relationship. Net photosynthesis was plotted for a leaf external [CO_2] equal to 350 ppm and saturating photon flux density (PFD).

To predict tree-ring Δ¹³C, we combined the slope values for canopy-level Δ¹³C from Fig. 6 and shifted the predicted intercept to minimize the sums of the squared errors across all tree-ring Δ¹³C measurements. The combined equation using both linear relationships was $\Delta^{13}C = -1.139 \times VPD - [0.0014 \times (PFD - 890.4)] + 21.40$, where 890.4 is the mean PFD across the five regions sampled. This combined relationship closely predicted the observed variation in Δ¹³C among regions and was not significantly different from the 1:1 line ($P = 0.693$), supporting the notion that VPD and light strongly influence tree-ring Δ¹³C for the oaks we sampled (Fig. 8; $\Delta^{13}C_{observed} = 1.05 \times \Delta^{13}C_{predicted} - 1.06$, $R^2 = 0.96$, $P = 0.003$).

Correlations between growth and Δ¹³C

Bur oaks from near the centre of their range in MO had RWI values that were positively related to Δ¹³C (Fig. 9; $r = 0.48$,

$P = 0.070$). The relationship was also positive, but stronger for SD oaks (Fig. 9; $r = 0.67$, $P = 0.003$) than those in MO. In contrast, for WI bur oaks at the northern edge of the species range, the relationship was significant but negative (Fig. 9; $r = -0.69$, $P = 0.001$). There was a negative relationship between these correlations and regional averages of 1/VPD or the ratio of precipitation to ET (Fig. 10) indicating that in dry regions growth and Δ¹³C were strongly positively correlated, but this relationship weakened in the central part of the bur oak range and approached zero or even became negative in the wet part of the range.

DISCUSSION

The growth of riparian bur oaks we investigated was very responsive to changes in VPD in regions where VPD was
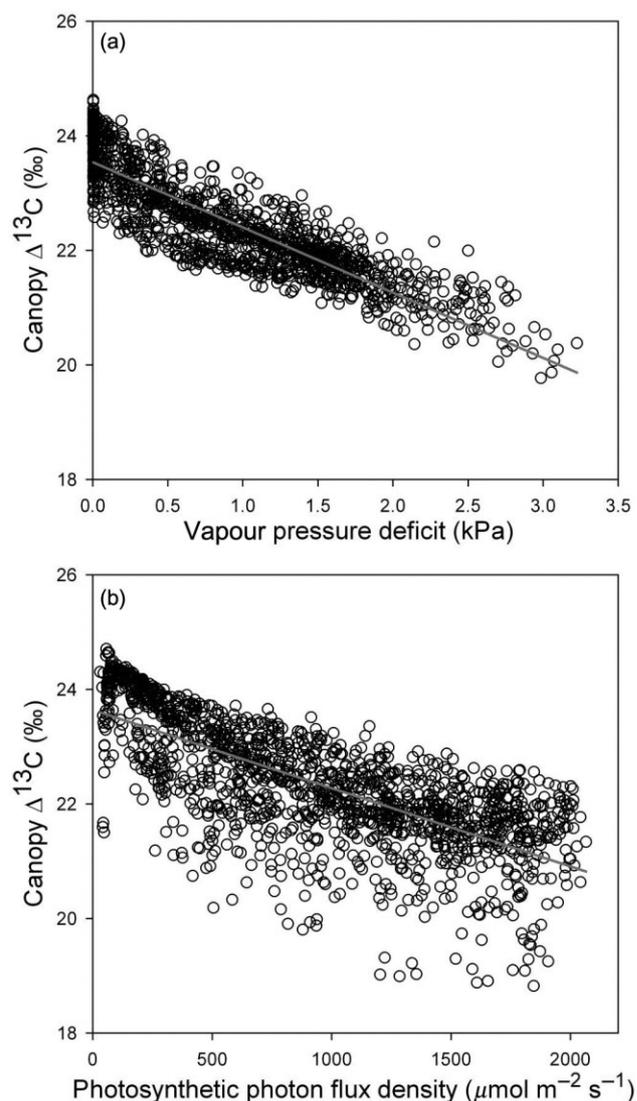

Figure 6. Canopy-integrated Δ¹³C estimated by the *CANISOTOPE* model versus vapour pressure deficit (a) and photosynthetic photon flux density (b) after Baldocchi & Bowling (2003).
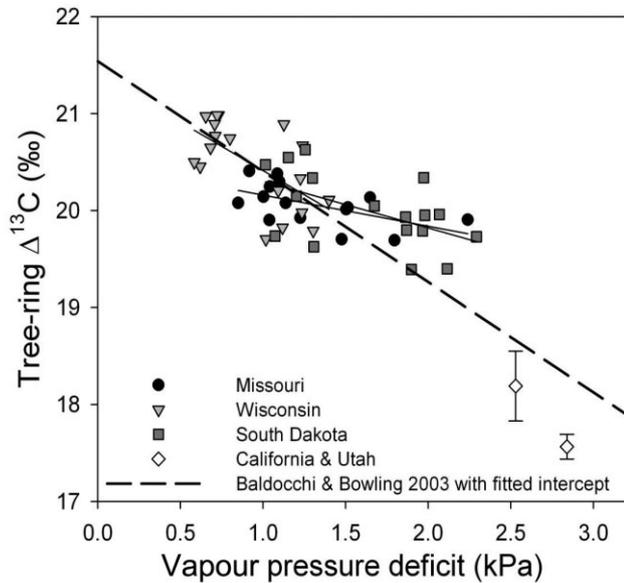
Figure 7. Tree-ring $\Delta^{13}C$ versus vapour pressure deficit (VPD) is shown by symbols across years for Missouri (MO), Wisconsin (WI) and South Dakota (SD). The symbols for California (CA) and Utah (UT) are means among trees (±1 SE). The dashed line is the canopy-level response (see Fig. 6a) with intercept fitted to the tree-ring $\Delta^{13}C$ data.

Figure 9. Ring-width index values versus tree-ring $\Delta^{13}C$ of bur oaks growing in three regions.

generally high but other factors became more influential where VPD was low. Therefore, controls on growth of these oaks may diverge from the principle of uniformity, making it difficult to interpret past variation in ring widths during
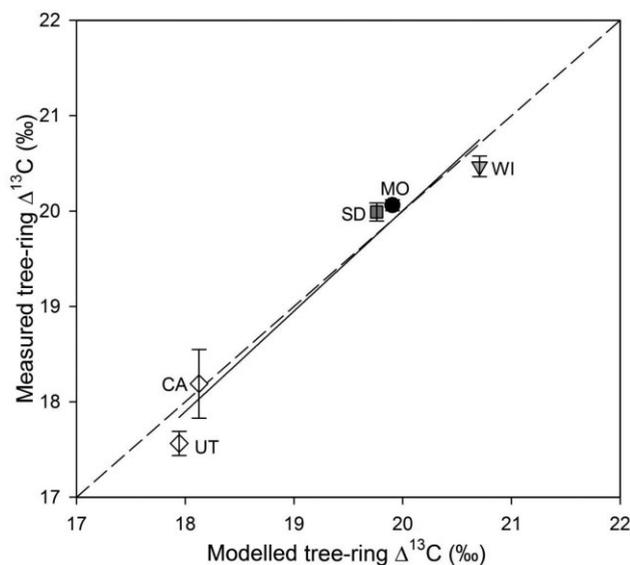
pluvial conditions. VPD also had a strong influence on tree-ring $\Delta^{13}C$, with regional differences in irradiance being of secondary importance. At the warmer and drier parts of the species range, RWI had positive correlations with tree-ring $\Delta^{13}C$, while this relationship was negative in the coolest and

Figure 8. Comparison of modelled and observed tree-ring $\Delta^{13}C$ (± 1SE) by region using growing season vapour pressure deficit (VPD) and photon flux density (PFD). The least squares linear relationship ($R^2 = 0.96$, $P = 0.003$) was not significantly different from the dashed 1:1 line ($P = 0.693$). $\Delta^{13}C$ was modelled using an equation that combined the responses of canopy-level $\Delta^{13}C$ to VPD and PFD (see the Methods and Results sections).

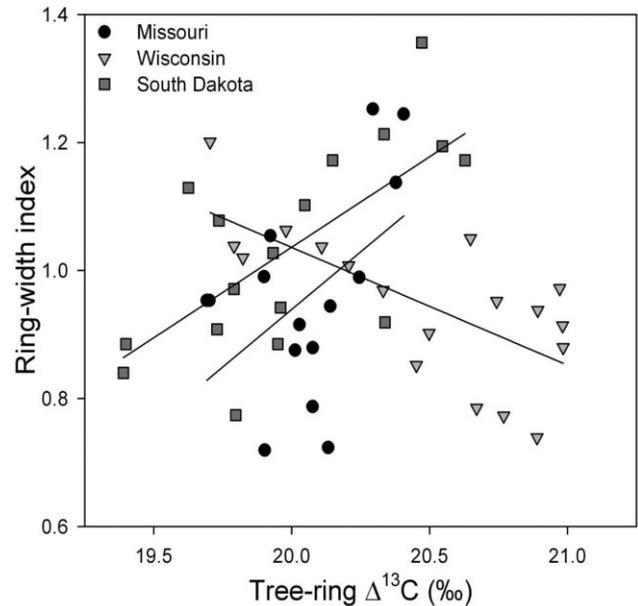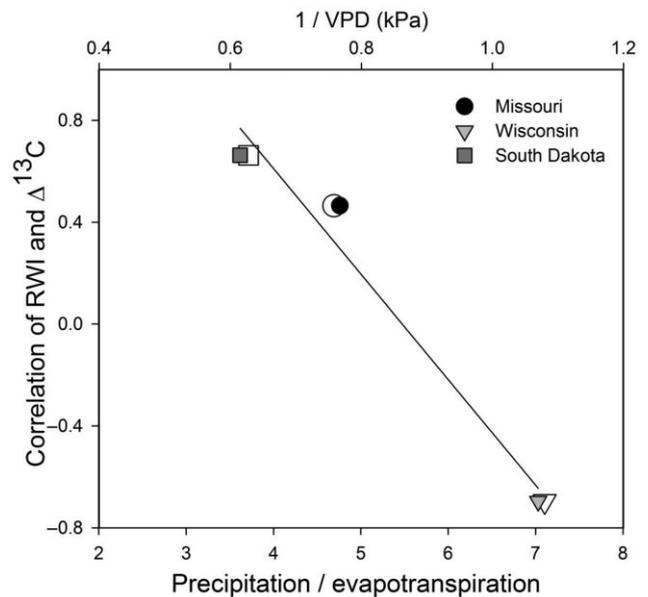**Figure 10.** Correlations of ring-width indices and tree-ring $\Delta^{13}C$ from each region scale with either of the ratio of precipitation to evapotranspiration (filled symbols) or 1/VPD (open symbols). Across this wetness gradient the trend line and labels indicate a continuum of changes in the relative influence of irradiance and moisture in limiting photosynthetic carbon gain and tree-ring growth.

wettest region sampled. Differences in the sign and magnitude of these correlations among regions scaled with 1/VPD or the ratio precipitation to ET, indicating the paired use of $\Delta^{13}C$ and ring widths can identify periods of past aridity versus wetness that would not be discernible by either of these signals used independently. Together, these results also suggest that the controls on carbon gain and growth shift between greater limitations by moisture versus irradiance across the range of bur oak.

Controls on growth across the bur oak range

Across three regions that encompass much of the bioclimatic envelope for bur oaks, inter-annual variation in growth was more strongly correlated with growing season VPD than with growing season precipitation (Figs 3 & 4) as we had anticipated for riparian trees. Traditionally, drought stress has been most consistently linked to inter-annual variation in radial growth (Stahle & Hehr 1984; Sieg *et al*. 1996; St. George & Nielsen 2002; Wyckoff & Bowers 2010; Stambaugh *et al*. 2011). In contrast, here we minimized the influence of soil moisture deficits by sampling riparian or well-watered trees to establish whether other factors that drive leaf-level physiology, also affect tree-ring growth and $\Delta^{13}C$ across the species range. Variation in growth responses due to stand history should have been minimized and have had little effect on correlations between RWI and meteorological data because we sampled trees from dominant or co-dominant canopy positions from multiple stands in each region. As expected for this shade intolerant species, bur oak tree rings offered little visual evidence of growth suppression and releases, indicative of past disturbances.

Correlations of RWI with growing season VPD and precipitation were weakest in WI, located at the northern edge of the species range (Fig. 3). These low correlations were not a function of the tree-ring series being complacent because the mean sensitivity and series inter-correlations were comparable to oaks at the dry edge of the range in SD (Table 1). Moreover, correlations adjusted for the range of VPD or precipitation showed similar rankings among regions (*data not shown*), indicating that the differences in the range of environmental variability had little influence on the patterns we show. Bur oaks sampled near rivers in the northern Great Plains of Manitoba, Canada, apparently responded to drought (St. George & Nielsen 2002), implying that for bur oaks in WI, latitude *per se* did not influence the correlations between RWI and VPD or precipitation. Correlations of RWI with growing season precipitation were higher for MO oaks than SD oaks. Closely related species of upland oaks (*Q. alba* L. and *Q. stellata* Wangenh.) have been characterized by radial growth that is more sensitive to drought or site water balance nearer to the western dry edge of the range (Stahle & Hehr 1984; LeBlanc & Terrell 2009; Goldblum 2010). The difference between our findings and previous trends across the species range likely owes to the ring widths of SD bur oaks being smaller and less variable compared to MO oaks, as reflected by their lower mean sensitivity and series inter-correlations (Table 1). In turn, these differences

reflect the rings of SD oaks being composed of a greater proportion of earlywood, derived from the use of non-structural carbon reserves stored during the previous growing season as earlywood forms during the spring before leaves are expanded (Voelker *et al*. 2012 *and references therein*). This notion is supported by RWI of SD bur oaks, but not those from MO or WI, showing significant correlations with the previous year's climate (*data not shown*), a finding that is often associated with upland oak species (Stahle & Hehr 1984; White *et al*. 2011). The correlations of RWI and VPD were similar for MO and SD bur oaks (Fig. 3). Indeed, once growing season length and irradiance regimes were accounted for, variability in the radial growth rates scaled similarly across regions with the growth of SD oaks being most clearly constrained during years with high VPD (Fig. 4).

Deeply rooted, riparian bur oaks presumably have adequate access to soil moisture during wet and dry years because they form a large tap root early in development (Weaver & Kramer 1932; Burns & Honkala 1990; Davis *et al*. 1999; Danner & Knapp 2001). In fact, stable isotope ratios of oxygen from water taken up by bur oaks indicate that trees growing at different distances from a river in Nebraska, USA, use deep soil water or ground water at 6–7 m depth for 70–88% of their transpiration and less than 8% from the top 0.3 m (Chimner & Resh, *in review*). Besides bur oak being deeply rooted, the influence of soil water deficits on tree-ring growth and stable isotope signals was further minimized by sampling trees located near streams or rivers. Thereby, our tree-ring data (Fig. 4) agree with model estimates (Fig. 7) and $\Delta^{13}C$ data suggesting that at the higher growing season VPDs occurring near the dry edge of the species range, carbon gain should be reduced at the canopy level through both leaf-level responses (Fig. 5) as well as diurnal losses of xylem conductance common in ring-porous species growing under well-watered conditions but exposed to high VPDs (Litvak *et al*. 2012).

If the VPD relationships described above were assumed to control growth of bur oaks, interpretations of paleo wood RWI such as those from Stambaugh *et al*. (2011) would be correct under a climate similar to or drier than that of the centre of the modern species range. However, the WI trees demonstrated that under cool and wet conditions, variations in RWI are less likely to represent differences in wetness or aridity, suggesting the assumption of a uniform climate response may not be appropriate for periods in which wetter conditions have prevailed. It is important to note that WI and SD bur oaks had similar mean sensitivity values and series inter-correlations, variables classically used by dendrochronologists to check the statistical quality as well as infer climate responsiveness of tree-ring chronologies (Fritts *et al*. 1965). Therefore, despite basic ring-width characteristics being outwardly similar, it would clearly be a mistake to use a modern proxy relationship to infer that trees growing in the distant past under SD-type climate conditions had ring-width variation being controlled by the same factors as those that grew under WI-type climate conditions. Our findings argue strongly for the use of additional proxy evidence, such as wood anatomy (Fonti *et al*. 2010; Voelker *et al*. 2012) or stable isotope signals (Young *et al*.

2010, 2012), to identify if certain periods have diverged from uniformity in growth controls.

VPD and irradiance predict variation in $\Delta^{13}C$

Factors influencing stomatal conductance or assimilation rate will, in turn, affect $\Delta^{13}C$. Among riparian or irrigated bur oaks that were largely decoupled from the effects of moderate soil moisture deficits, our working hypothesis was that VPD and irradiance were the main variables that influence stomatal conductance, assimilation rates and thus $\Delta^{13}C$. We recognize that $\Delta^{13}C$ can be affected by local environments, individual tree attributes as well as fractionation processes that the original Farquhar model (Farquhar *et al.* 1982) did not consider. We address these issues below, but, altogether, these factors should be minimal for the species, conditions and sampling design employed in this study.

For this study, we tested whether canopy-level $\Delta^{13}C$ variation related to VPD and PFD estimated by Baldocchi & Bowling (2003) for an oak-dominated forest across a growing season could be used to predict $\Delta^{13}C$ of oak tree rings sampled across regions differing in those same variables. Across inter-annual and regional variation, VPD and PFD levels should be positively correlated. All else equal, this co-variation and potential interaction could result in estimations of the $\Delta^{13}C$ response to either of VPD or PFD to be stronger than if they could be estimated independently. However, for a given level of PFD, canopy-level photosynthesis increases as cloudiness and the fraction of diffuse light increase (Roderick *et al.* 2001 *and references therein*; Still *et al.* 2009; Yang *et al.* 2010). Hence, if diffuse light occurs less frequently at higher VPD or PFD levels, the true relationship of canopy $\Delta^{13}C$ to VPD would not be as steep as measured. To the extent that these effects cancel each other out, the relationships of Baldocchi & Bowling (2003) should be accurate as general responses to VPD and PFD for oaks. Indeed, there was close agreement between measured and modelled $\Delta^{13}C$ values after we accounted for differences in the intercepts between the canopy-integrated responses versus our tree-ring data (Fig. 8).

In some cases, soil nutrient status has been shown to affect $\Delta^{13}C$ after fertilization through increases in the ratio of photosynthesis to stomatal conductance (Clearwater & Meinzer 2001; Brooks & Coulombe 2009; Cernusak, Winter & Turner 2009a; Brooks & Mitchell 2011). However, data from a number of other studies have not demonstrated the expected decreases in tree-ring $\Delta^{13}C$ in response to nitrogen fertilization or nitrogen deposition (Elhani *et al.* 2005; Betson *et al.* 2007; Balster, Marshall & Clayton 2009; Talhelm, Pregitzer & Burton 2011; Krause *et al.* 2012; Leonardi *et al.* 2012). These mixed results may result from some sites not being very nitrogen-limited and/or because in response to large doses of fertilizer, leaf nitrogen content may only be elevated over relatively short time frames before returning to pre-treatment levels (Brix 1971; Brooks & Mitchell 2011). Moreover, plant $\Delta^{13}C$ may only respond to soil fertility on soils that are also prone to drought effects as well (Högberg, Johannisson & Hallgren 1993; Korol *et al.* 1999). We did not

sample soils for nutrient analyses. However, because the trees sampled in MO, SD and WI were all growing on sites with consistent water availability and relatively rich alluvial or colluvial soils that should not be highly nutrient-limited, we consider this potential influence on tree-ring $\Delta^{13}C$ to have been minor.

For the trees located in UT and CA, we sampled branch wood rather than bole wood. There are many reports of gradients in $\delta^{13}C$ between leaves and stems of C_3 plants, but differences with distance from source tissues are often relatively small and vary among species and times during the growing season, making it difficult to discern whether branch wood will differ from trunk wood due to post-photosynthetic fractionation (Cernusak *et al.* 2009b). If this pattern did influence our data, $\Delta^{13}C$ of wood in UT and CA would have been slightly less had we sampled boles rather than branches. Compared with the effects of VPD and PFD, this difference would have been small and could not have substantially altered the fitted relationship between measured and modelled $\Delta^{13}C$ (i.e. Fig. 8).

Correlations between growth and $\Delta^{13}C$ differ across the bur oak range

For our study, correlations between RWI and meteorological variables did not strongly differentiate RWI chronologies from MO and SD (Fig. 3) despite substantial differences in precipitation and VPD (Table 1). However, the patterns in RWI_a and the stronger positive correlation between RWI and $\Delta^{13}C$ for SD than for MO reveal that leaf-level physiology and variability in growth were more constrained by moisture stress at SD than MO (Figs 4 & 9). In contrast, correlations between RWI and $\Delta^{13}C$ for trees from WI were negative (Fig. 9), implying assimilation rates of WI bur oaks were more constrained by lower PFD or wetter conditions. It is unlikely that reduced stomatal conductance and assimilation associated with dry growing seasons directly favoured assimilation and growth of oaks in WI. However, PFD and daytime canopy temperatures should be greater during relatively dry summers with fewer clouds, making inter-annual variation in irradiance the most important determinant of assimilation and growth at this relatively cool and wet location. Similarly, in Norway, a $\Delta^{13}C$ chronology was used to infer past patterns in irradiance, and when paired with a nearby tree-ring-based temperature reconstruction, the combination of signals indicated cycles when these conditions were decoupled in association with shifts in atmospheric circulation (Young *et al.* 2010, 2012).

Although the average growing season precipitation is similar between MO and WI (Table 1, Fig. 4), the cooler temperatures in WI result in lower VPD and ET. This results in the correlation between RWI and $\Delta^{13}C$ scaling similarly with either of 1/VPD or the ratio of precipitation to ET (Fig. 10). Therefore, we propose that RWI and $\Delta^{13}C$ chronologies can be used to produce running correlations diagnostic of when oak carbon gain and growth are more moisture limited versus irradiance limited. Indeed, the trend in correlation strength and direction noted here are part of a larger trend across

species where $\Delta^{13}C$ and RWI series have strong correlations for trees growing in semi-arid regions (Mazany *et al.* 1980; Leavitt *et al.* 2010), but weaker correlations for trees growing in cooler and wetter regions (Tans & Mook 1980; Brooks, Flanagan & Ehleringer 1998; Kirdyanov *et al.* 2008; S. Voelker, *unpublished data*).

In conclusion, the control over RWI of bur oaks by climatic factors within a region differed greatly across continental gradients in precipitation, vapour pressure deficit and irradiance. As such, for ring-width variation in this species, the principle of uniformity cannot be assumed to apply to all periods, particularly during cold and wet conditions. Across regions, $\Delta^{13}C$ and growth of bur oaks respond strongly to variability in VPD and to a lesser extent, PFD. Using *a priori* relationships between canopy $\Delta^{13}C$ and either of VPD and PFD (i.e. Baldocchi & Bowling 2003), we could closely predict tree-ring $\Delta^{13}C$ of bur oaks. Finally, we showed that the magnitude and sign of correlations between RWI and $\Delta^{13}C$ chronologies corresponded to regional shifts in VPD or precipitation/ET. In combination with paleo wood resources (Stambaugh *et al.* 2011; Voelker *et al.* 2012), these signals should prove valuable for inferring shifts in past atmospheric circulation and climatic cycles.

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