Elevated CO_2 increases tree-level intrinsic water use efficiency: insights from carbon and oxygen isotope analyses in tree rings across three forest FACE sites

Giovanna Battipaglia[1,2], Matthias Saurer[3], Paolo Cherubini[4], Carlo Calfapietra[5], Heather R. McCarthy[6], Richard J. Norby[7] and M. Francesca Cotrufo[8]

[1]Environmental Science Department, Second University of Naples, 81100, Caserta, Italy; [2]Centre for Bio-Archeology and Ecology, Ecole Pratique des Hautes Etudes (PALECO EPHE), Institut de Botanique, University of Montpellier 2, F–34090, Montpellier, France; [3]PSI Paul Scherrer Institute, 5232, Villigen, Switzerland; [4]WSL Swiss Federal Institute for Forest, Snow and Landscape Research, 8903, Birmensdorf, Switzerland; [5]IBAF-Institute of agro-environmental and Forest Biology, CNR, Porano, Italy; [6]Department of Microbiology and Plant Biology, University of Oklahoma, Norman, OK, USA; [7]Oak Ridge National Laboratory, Environmental Sciences Division, Oak Ridge, TN, USA; [8]Department of Soil and Crop Sciences, Colorado State University, Fort Collins, CO, USA

Summary

- Elevated CO_2 increases intrinsic water use efficiency (WUE_i) of forests, but the magnitude of this effect and its interaction with climate is still poorly understood.
- We combined tree ring analysis with isotope measurements at three Free Air CO_2 Enrichment (FACE, POP-EUROFACE, in Italy; Duke FACE in North Carolina and ORNL in Tennessee, USA) sites, to cover the entire life of the trees. We used $\delta^{13}C$ to assess carbon isotope discrimination and changes in water-use efficiency, while direct CO_2 effects on stomatal conductance were explored using $\delta^{18}O$ as a proxy.
- Across all the sites, elevated CO_2 increased ^{13}C-derived water-use efficiency on average by 73% for *Liquidambar styraciflua*, 77% for *Pinus taeda* and 75% for *Populus sp.*, but through different ecophysiological mechanisms.
- Our findings provide a robust means of predicting water-use efficiency responses from a variety of tree species exposed to variable environmental conditions over time, and species-specific relationships that can help modelling elevated CO_2 and climate impacts on forest productivity, carbon and water balances.

Introduction

Terrestrial plants play a significant role in the global carbon cycle and in the control of the carbon dioxide (CO_2) concentration in the atmosphere. The responses of plants to an increasing concentration of atmospheric CO_2 will depend on their ability to use water and nutrient resources efficiently under a changing climate. While the potential growth response of trees to increased atmospheric CO_2 concentration is relatively well understood, there is still uncertainty regarding the process and environmental constraints that modulate the responses of photosynthesis and stomatal conductance to elevated CO_2 (Wullschleger *et al.*, 2002; Herrick *et al.*, 2004; Ainsworth & Long, 2005; Keel *et al.*, 2006), and the tree-level expression of those responses as intrinsic water use efficiency (WUE_i: the amount of carbon acquired per unit of water lost) in the long term (Ainsworth & Rogers, 2007; Gagen *et al.*, 2011).

Elevated atmospheric CO_2 (c_a) is expected to affect plant carbon–water relationships, as a decline in stomatal conductance (g_s) is predicted when plants are exposed to elevated CO_2 (Field

et al., 1995). If a decline in g_s occurs in conjunction with an increase in carbon assimilation (owing to CO_2 supply), this can change the c_a to internal leaf CO_2 concentration (c_i) gradient, which in turn determines the ^{13}C isotope composition of assimilated carbon. it is known that discrimination against ^{13}C ($\Delta^{13}C$) during photosynthesis in C3 plants is related to the c_i/c_a ratio, where the c_i/c_a level is determined by the relationship between photosynthesis (A) and g_s ($WUE_i = A/g_s \sim c_a$-c_i). As the stomata tend to close under elevated CO_2, the mechanism for saving water often results in an improvement of the WUE_i. When the stomatal limitation of photosynthesis is strong, the plants exhibit a low c_i and a reduced discrimination, along with improved WUE_i (McCarroll & Loader, 2004; Saurer *et al.*, 2004). Thus, discrimination against ^{13}C is a proxy for the integrated response of A and g_s, and can be used to infer average plant WUE_i over the time when the plant organic matter was formed. In tree rings, carbon-isotope based WUE-estimates reflect a growing season (photosynthesis-weighted) average, that is difficult to assess with instantaneous gas-exchange measurements (Picon *et al.*, 1997).

The relative oxygen isotope ratio ($\delta^{18}O$) in tree rings can provide a different insight into the causes of variation of WUE_i. It has been suggested that $\delta^{18}O$ in tree rings can be used as a proxy for g_s (Farquhar *et al.*, 1993; Barbour *et al.*, 2000). Variations in g_s influence the $\delta^{18}O$ of plant material through different competitive effects on: (1) the evaporative cooling of the leaf; (2) the diffusive resistance of water vapour; and (3) the plant transpiration and the subsequent gradient in $H_2^{18}O$ enrichment in leaf water, also known as the Péclet effect (Barbour *et al.*, 2004). When g_s decreases under elevated CO_2 and photosynthetic CO_2 demand is unchanged, c_i/c_a should decrease and $\delta^{18}O$ in leaf water, as well as in the organic matter and tree rings, would increase (Roden & Ehleringer, 2000).

The combined analyses of carbon and oxygen isotopes in tree rings can therefore suggest whether g_s or A contribute most to the variation of WUE_i in response to elevated CO_2 (Scheidegger *et al.*, 2000).

Several studies, using $\Delta^{13}C$ in trees growing in natural conditions, have shown wide variability among forests species in their WUE_i responses to rising c_a (Penuelas *et al.*, 2011 and references therein). In most cases WUE_i was improved, but there is a lack of understanding of the extent to which the elevated CO_2 increased WUE_i of different species and which mechanism (i.e. stomatal closure or A increases) drove the differences (or similarities) between WUE_i in the various species and sites (Gagen *et al.*, 2011; Linares & Camarero, 2012). Free-air CO_2 enrichment (FACE) field experiments have played a key role in addressing uncertainties in forest responses to elevated c_a (reviewed in Ainsworth & Long, 2005), but with contrasting results regarding the principal mechanisms involved in the increase in plant WUE_i (Norby & Zak, 2011).

Previous studies (Table 1) have reported reduced g_s in response to elevated CO_2, but the magnitude of the response varied widely across sites and species (Warren *et al.*, 2011a,b) and appeared to be influenced by other environmental and growth-related factors (Leakey *et al.*, 2009; Norby & Zak, 2011). Those studies

estimated the responses of stomatal conductance to rising CO_2 from short-term, leaf 'exposure' analysis, which cannot take account of acclimatization that may alter stomatal responses over longer time-scales (Gagen *et al.*, 2011). Analyses of tree-rings are ideal proxies for assessing physiological and growth-changes over time and under different environmental conditions and allow assessment of pretreatment behaviour.

This study examines the temporal relationships between variations in tree ring $\delta^{13}C$-derived WUE_i and $\delta^{18}O$ with c_a, climate and tree growth, across five species in three FACE sites in Italy and the USA. They represent a wide range of climate, biological conditions, stand development history and dominant tree species, with a broadleaved species, *Liquidambar styraciflua*, growing in two FACE sites (ORNL and Duke), a coniferous species, *Pinus taeda*, growing in a mixed fumigated forest (Duke) and three fast-growing poplar species (*Populus sp.*), planted in a Mediterranean environment. Hence, they offered us the possibility to test the following hypotheses: (1) the mechanism driving the expected improvement in WUE_i under elevated CO_2 is more strongly related to species than to site conditions; (2) CO_2 fertilization will interact with climate controls on WUE_i, influencing tree responses to temperature and precipitation; (3) the combined tree-ring width, carbon and oxygen isotope analysis enables us to understand why increased WUE_i sometimes does not lead to increased growth. To test those hypotheses, we applied a dendro-ecological approach at three FACE sites to investigate whether any observed patterns extend across species, and we analysed *Liquidambar styraciflua*, present in two different sites, to assess whether CO_2 responses are site dependent. Further, we analysed tree rings of individual trees before and after the beginning of FACE experiments to test for legacy effects (i.e. translation of trees before treatment condition and life history) into responses to elevated CO_2.

The simultaneous measurements of isotopes and growth allow us to estimate species-specific changes in WUE_i and can be useful to predict the influence of future climate change on the productivity and water consumption of major trees used in forest plantations.

Table 1 Summary results of the effects of elevated CO_2 for different sites regarding net primary productivity (NPP), stomatal conductance (g_s) and photosynthetic activity (A)

Site	Species	NPP	g_s	A
ORNL	L. s	+24% (2001–2003) + 9 (2008)[1]	−14–44% (1999–2000)[2]	+44% (1999–2000)[3] nc (2008–2010)[1]
Duke	L. s	nr	−28% (1998–2001)[4]	+41–52%[5]
Duke	P. t	+22–30% (1997–2004)[6]	nc[7]	+35–65%[7]
POP-EUROFACE	P. a; P. n; P. e	+21–26%[8]	−19 to 24%[9]	+38%[10]

Responses are indicated as increases (+), decreases (−) no change (nc) or no reported information (nr). L. s, *Liquidambar styraciflua*; P. t, *Pinus taeda*; P. a, *Populus alba*; P. n, *Populus nigra*; P. e, *Populus x euramericana*.
[1]Norby *et al.* (2010).
[2]Gunderson *et al.* (2002).
[3]Sholtis *et al.*, (2004).
[4]Herrick *et al.* (2004).
[5]Springer & Thomas (2007).
[6]McCarthy *et al.* (2010).
[7]Ellsworth *et al.* (2012).
[8]Gielen *et al.*, (2005).
[9]Tricker *et al.* (2005).
[10]Bernacchi *et al.*, (2003) .

Materials and Methods

Site descriptions

Three FACE experiments in fast-growing temperate-zone tree plantations were sampled for our study. In particular, two experiments were in established monoculture plantations: a deciduous sweetgum (*L. styraciflua* L.) stand at ORNL (Oak Ridge National Laboratory) FACE, Tennessee (USA) and an evergreen loblolly pine (*P. taeda* L.) stand at Duke FACE, North Carolina (USA). The third site was a poplar plantation (*Populus alba* L., *Populus nigra* L., *Populus x euramericana* Dode Guiner), established on a former agricultural field, in Tuscania (Italy).

All three experimental sites used FACE technology and full detailed descriptions are provided for Duke FACE by Hendrey *et al.* (1999), for ORNL FACE by Norby *et al.* (2001) and for the POP-EUROFACE by Miglietta *et al.* (2001).

In the ORNL site the *L. styraciflua* plantation was established in 1988 and the CO_2 fumigation started in 1998. Our data refer to two fumigated and two control plots and cover the period 1988–2004.

The Duke FACE site was established in a mixed forest containing an overstory of *P. taeda* planted in 1983 and several deciduous understory trees, including *L. styraciflua*, *Acer rubrum*, *Ulmus alata*, *Cercis canadensis* and *Cornus florida*. The fumigation started in 1996 and our data covers the period 1983–2004.

In the POP-EUROFACE the poplar plantation (*P. alba*, *P. nigra* and *P. x euramericana)* and the FACE facility were established in 1999, coppiced in 2001 and the FACE experiment ended in 2004. For this study we analysed the tree rings from stems grown during the second coppiced rotation (2002–2004), including the growing season after the end of the fumigation (2005).

Full description of the sites and of the atmospheric CO_2 enrichment supply is provided in the Supporting information, Notes S1.

Meteorological data

We used the climate data reported by Riggs *et al.* (2003) for the ORNL FACE (Fig. S1), which measured at the site since April 1997 (http://face.env.duke.edu/database/login.cfm) for the Duke FACE (Fig. S2), and that recorded by the site meteorological station for the POP-EUROFACE (Fig. S3). Meteorological data were also obtained from the Southeastern Regional Climate Center of the Department of Natural Resources (http://www.sercc.com/sco) and from the Climate Explorer (http://climexp.knmi.nl) to calculate monthly anomalies of precipitation and temperature for the duration of the experiment relative to long-term mean values (1900–2009) (see Notes S2, Figs S1–S3).

Tree core sampling

Eight trees of each species per each experimental plot were sampled during a field campaign in the USA and Italy in spring–summer 2005. At the ORNL FACE and at the POP-

EUROFACE overstory trees were cored. At the POP-EURO-FACE site, characterized by a multistem system during the second rotation cycle, dominant shoots on each stump were sampled. For DUKE FACE, we sampled the overstory *P. taeda* as well as understory *L. styraciflua*. Two cores were collected with a 5 mm diameter borer (Suunto, Finland), near the base of each tree, following standard methods (Schweingruber, 1988).

Tree ring width

Ring-width measurements were made with a resolution of 0.01 mm on each of the cores, using LINTAB measurement equipment (Frank Rinn, Heidelberg, Germany) fitted with a Leica MS5 stereoscope and analysed with TSAP software package. All cores were cross-dated according to standard procedure (Fritts, 1976; Schweingruber, 1996). We derived the series of yearly basal area increments (BAI) from raw ring width assuming concentrically distributed tree-rings; BAI were used instead of ring-width directly, because BAI is less dependent on age and thus avoids the need for detrending (Biondi, 1999), which could also remove low frequency variability.

Isotope analysis

Tree rings from all cores were split into earlywood and latewood, and only latewood was used in this study (Lipp *et al.*, 1991; Hill *et al.*, 1995). The latewood of the two cores per tree was pooled by year, and the samples were ground with a centrifugal mill (ZM 1000, Retsch, Germany) using a mesh size of 0.5 mm to ensure homogeneity. Cellulose was extracted with a double-step digestion (Boettger *et al.*, 2007; Battipaglia *et al.*, 2008). The carbon and oxygen stable isotope compositions were measured at the CIRCE laboratory (**C**enter for **I**sotopic **R**esearch on the **C**ultural and **E**nvironmental heritage, Caserta, Italy) and at the PSI isotope-lab (Villigen, Switzerland), respectively, by continuous-flow isotope ratio mass spectrometry (Finnigan Mat, Delta S, Bremen, Germany) using 1 mg of dry matter for ^{13}C measurements and 1.5 mg for ^{18}O determinations.

We report isotope values in the delta notation for carbon and oxygen, where $\delta^{13}C$ or $\delta^{18}O = (R_{sample}/R_{standard} - 1)$ (‰), relative to the international standard, which is VPDB (Vienna Pee Dee Belemnite) for carbon and VSMOW (Vienna Standard Mean Ocean Water) for oxygen. R_{sample} and $R_{standard}$ are the molar fractions of $^{13}C/^{12}C$ and $^{18}O/^{16}O$ for the sample and the standard, respectively. The standard deviation for the repeated analysis of an internal standard (commercial cellulose) was better than 0.1‰ for carbon and 0.2‰ for oxygen. The calibration vs VPDB was done by measurement of International Atomic Energy Agency (IAEA) USGS-24 (graphite) and IAEA-CH7 (polyethylene) and vs VSMOW by measurement of IAEA-CH$_3$ (cellulose) and IAEA-CH$_6$ (sucrose).

Data analysis

As the series of consecutive observations are collected from the same tree (annual rings from an increment core), data

autocorrelation may arise (Monserud & Marshall, 2001). Thus, to determine and remove long-term trends without altering or modifying the existing data, the response ratio (RR, the ratio of means for a measured variable between the elevated CO_2 treatment group and the control group) was used as an index of the estimated magnitude of the elevated CO_2 effect. The RR is commonly used as a measure of experimental effects or manipulations (Hedges *et al.*, 1999) and has often been used in ecology to test the response of biomass or nitrogen (N) concentrations to increased CO_2 levels (Gunderson & Wullschleger, 1994; Cotrufo *et al.*, 1998).

For c_i/c_a, WUE_i and $\delta^{18}O$, the statistical analyses were conducted on the natural logarithm of the two parameters:

$$\ln(RR) = \ln \frac{X_1}{X_0} \qquad \text{Eqn 1}$$

where, for the response to elevated atmospheric CO_2, X_1 corresponds to values of ring width and $\delta^{18}O$ in FACE plots, and X_0 to the respective value in controls plots. Log-transformed data were used in order to linearize the metrics and to have a normal distribution even with a small number of samples (Hedges *et al.*, 1999).

For ^{13}C we could not directly use the $\delta^{13}C$ of control and FACE wood, because the CO_2 used at DUKE, ORNL FACE and POP_EUROFACE was depleted in ^{13}C (Notes S1), while the $\delta^{13}C$ ratio of the background atmospheric CO_2 is c. $-8.0‰$. To account for these differences in the source isotope signal, ^{13}C isotope discrimination ($\Delta^{13}C$) was calculated, according to Farquhar *et al.* (1982):

$$\Delta^{13}C = \frac{(\delta a - \delta m)}{(1 + \delta m)} \qquad \text{Eqn 2}$$

where $\delta m = \delta^{13}C$ of the tree ring and $\delta a = \delta^{13}C$ of the atmospheric CO_2. The RR was calculated from those values.

In FACE plots, for the years after the start of fumigation, eqn (2) becomes:

$$\Delta^{13}C = \frac{(\delta x - \delta m)}{(1 + \delta m)}$$

where $\delta x = \delta^{13}C$ of atmospheric CO_2 in FACE plots and the following mass balance was applied to calculate δ_x:

$$\delta_x = \frac{\delta_a [CO_2]_a + \delta_f [CO_2]_F}{[CO_2]_x}$$

where $[CO_2]_x$ is the total concentration of CO_2 in each fumigated plot, δ_a and $[CO_2]_a$ is, respectively, the $\delta^{13}C$ and the concentration of atmospheric CO_2 and δ_F, and $[CO_2]_F$ is the ^{13}C value and concentration of CO_2 used for fumigation. $[CO_2]_F$ was the growing season mean of the continuous series of CO_2 concentration available online for each plot at each site (http://public.ornl.gov/face/ORNL/ornl_data_co2weather.shtml for ORNL and http://face.env.duke.edu/database/for Duke). We used the

equations above to calculate discrimination at all sites and plots. Plant $\Delta^{13}C$ values are then used to estimate WUE_i using the following two equations (Farquhar *et al.*, 1989):

$$\Delta^{13}C = a + (b - a) * (c_i/c_a)$$

where a is the fractionation resulting from diffusion (4.4‰), and b is the fractionation associated with carboxylation by Rubisco (c. 27‰). Note that $\Delta^{13}C$ should be directly related to the CO_2 concentration in the chloroplast (c_c) rather than c_i. As a result, using c_i may create complications if mesophyll conductance to CO_2 is not constant (Seibt *et al.*, 2008). WUE is estimated from c_i and c_a as follows:

$$WUE_i = A/g_s = (c_a - c_i)/1.6$$

where 1.6 is the ratio of diffusivities of water and CO_2 in air. A/g_s values estimated here are strongly dependent on the parameter assumptions of the model and that mesophyll conductance does not limit A.

BAI, $\Delta^{13}C$ and $\delta^{18}O$ raw data are reported in Figs S4, S5.

For statistical analysis, BAI, $\Delta^{13}C$ and $\delta^{18}O$ measurements were normalized to account for pretreatment differences by subtracting each tree's pretreatment mean value from the values for each year in the tree's time-series. Data across years, period of fumigation and treatments were analysed by a nested analysis of variance. Species and CO_2 treatments were tested at a significance level of $P < 0.05$.

Partial correlations between RR_WUE_i vs $\delta^{18}O$ and climate variables were calculated to identify the climate parameters that best explained variation in tree growth and physiology. Partial correlation is a procedure that allows us to better understanding the relationship between variables (Kleinbaum *et al.*, 2008).

Pearson and correlation coefficients were calculated using precipitation, temperature and soil moisture data from each site. Linear regressions were performed in order to explore the relationship between WUE_i vs the other parameters.

Correlation, regression and time-series analyses were carried out using the SPSS 16.0 statistical package (SPSS, Chicago, IL, USA).

Results

FACE effects and temporal dynamics of c_i/c_a and WUE_i

Measurement of carbon isotopes indicated a significant decrease in c_i/c_a in all the fumigated plots compared with controls for all the tree species analysed (Figs 1, 2). The declining trend in c_i/c_a at ORNL and DUKE started with the start of the FACE treatment and reached minimum values for *L. styraciflua* growing at Duke during 1996–2002 (Fig. 1b). Post-fumigation measurements were taken at the POP-EUROFACE in 2005, and they showed that there were no longer significant differences in c_i/c_a between post FACE-treatment and control plots (Fig. 2a–c).

A novel component of our work is the analysis of physiological characteristics of trees at Duke and ORNL FACE and control
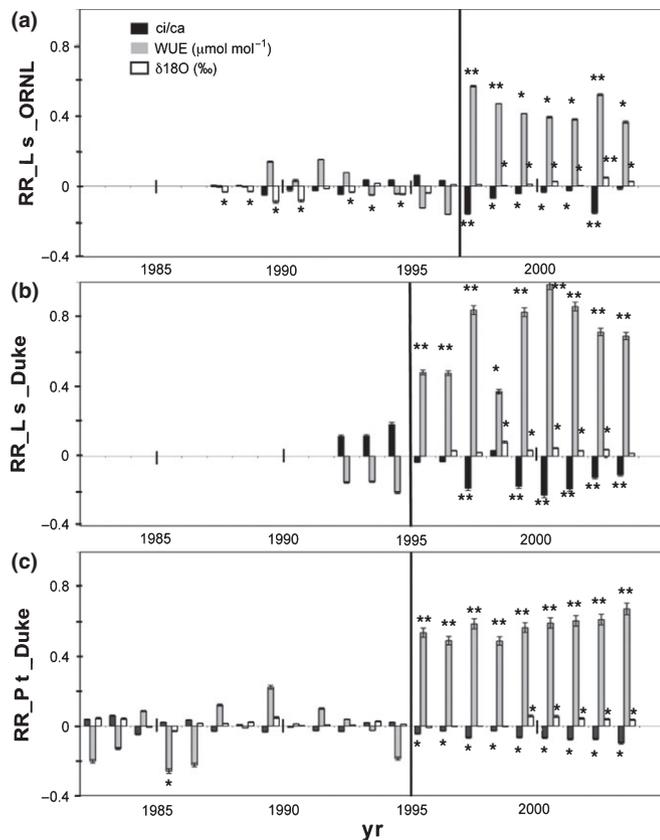
Fig. 1 Natural logarithm of response ratio (RR) of c_i/c_a (closed bars), intrinsic water use efficiency (WUE)$_i$ (tinted bars), $\delta^{18}O$ (open bars) related to *Liquidambar styraciflua* at ORNL (a), *L. styraciflua* (b) and *Pinus taeda* (c) at Duke. The vertical lines point to the beginning of fumigation for ORNL (1998) and Duke FACE (1996). Bars are standard deviation ($n = 8$). Statistical significance is indicated as: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$. RR_L. s_ORNL, response ratio of *Liquidambar styraciflua* at ORNL; RR_L.s_DUKE, response ratio of *Liquidambar styraciflua* at DUKE FACE; RR_P. t_Duke, response ratio of *P. taeda* at DUKE FACE.

Fig. 2 Natural logarithm of response ratio (RR) of c_i/c_a (closed bars), intrinsic water use efficiency (WUE)$_i$ (tinted bars), $\delta^{18}O$ (open bars) related to *Populus alba* (a), *Populus nigra* (b), *Populus x euramericana* (c) at POP-EUROFACE. The vertical lines point to the end of fumigation for POP-EUROFACE (2005). Bars are standard deviation ($n = 8$). Statistical significance is indicated as: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$. RR_P. a, response ratio of *Populus alba*; RR_P. n, response ratio of *Populus nigra*; RR_P. e, response ratio of *P. x euramericana*.

Table 2 Intercellular partial pressure of CO_2 (c_i) and atmospheric CO_2 (c_a) for elevated (c_i/c_a_E) and control (c_i/c_a_C) plots with the corresponding per cent increase during the fumigation period analysed

Site	Species	c_i/c_a_E	c_i/c_a_C	c_i%	c_a%
ORNL	L. s	0.57	0.63	+25	+39
Duke	L. s	0.63	0.70	+35	+50
Duke	P. t	0.70	0.75	+41	+50
POPEUROFACE	P. a	0.63	0.65	+53	+57
POPEUROFACE	P. n	0.70	0.72	+53	+57
POPEUROFACE	P. e	0.70	0.75	+45	+57

Data are for 1998–2004 for ORNL; 1996–2004 for Duke and 2002–2004 for POPEUROFACE. L. s, *Liquidambar styraciflua*; P. t, *Pinus taeda*; P. a, *Populus alba*; P. n, *Populus nigra*; P. e, *Populus x euramericana*.

plots before the beginning of the FACE study. Before fumigation started, c_i/c_a was not statistically different in control and experimental plots, ranging, on average, between 0.65 and 0.78 for all plots.

Based on our estimates of c_i/c_a, we calculated c_i at ambient and elevated atmospheric CO_2, which increased under elevated CO_2 in all tree species (Table 2).

Similarly, in all the experimental years and at each site, we observed a pronounced and significant increase in WUE$_i$ under elevated CO_2, compared with control. In the POP-EUROFACE site, the increase in WUE$_i$ was reduced but still present in 2005, a year after the end of fumigation (Fig. 2). The linear regression (Fig. 3a) between WUE$_i$ in control plots (WUE_C) and WUE$_i$ in fumigated plots (WUE_E) during the CO_2 exposure was largely consistent across the broad range of species and sites, with an average WUE$_i$ increase of 75 ± 13% under elevated CO_2. The linear regression is above the 1 : 1 line ($P < 0.001$) indicating a significant stimulation effect of CO_2 on WUE$_i$. The coefficient of determination (r^2) is 0.55, $P < 0.01$, the slope is significantly < 1 and the intercept is significantly different from 0 and positive,
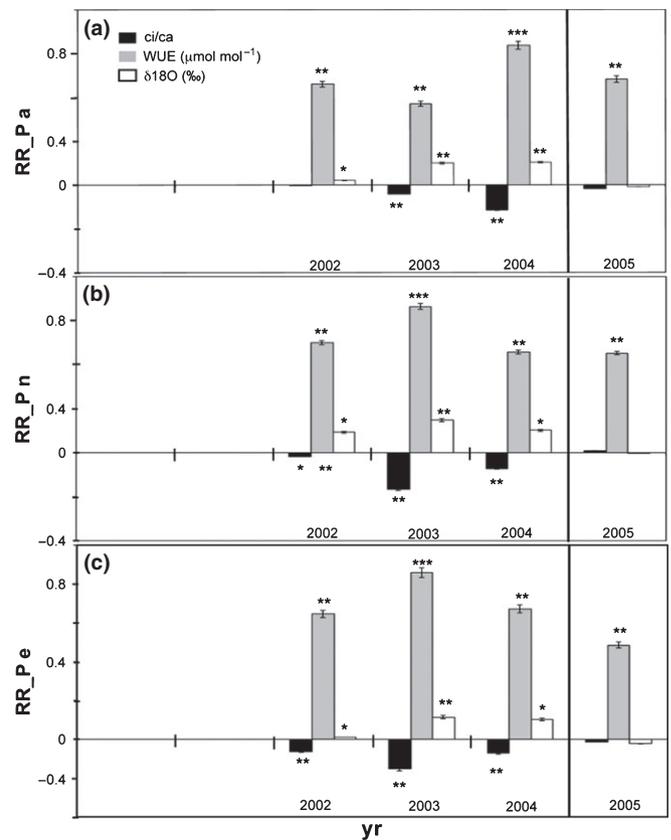
showing that, overall, as WUE$_i$ increases the CO_2 stimulation declines.

FACE effects and temporal dynamics of $\delta^{18}O$

For *L. stryraciflua* growing at ORNL, in the 9 yr before the FACE experiments, the $\delta^{18}O$ of tree rings in the future FACE plots were
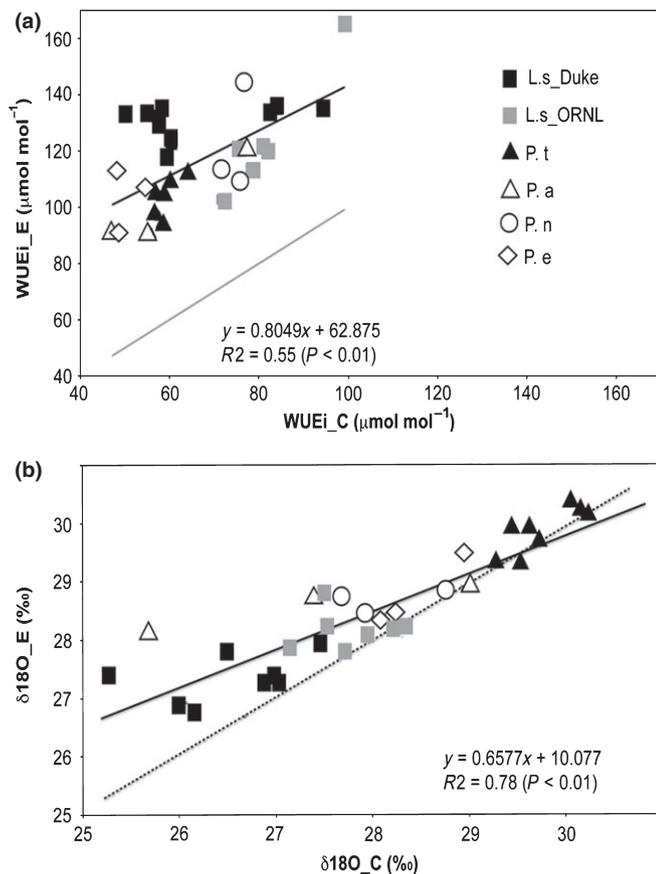
Fig. 3 Regressions of intrinsic water use efficiency (WUE$_i$)_E (a) and $\delta^{18}O$_E (b) as a function of WUE$_i$_C and $\delta^{18}O$_C, respectively. The dotted line represents the 1 : 1 relationship. Each data point represents the mean value under elevated CO_2 and control sites for each species investigated during the fumigation period: 1998–2004 for ORNL, 1996–2004 for Duke and 2002–2004 for POP-EUROFACE. L. s_Duke, *Liquidambar styraciflua* at DUKE; L. s_ORNL, *Liquidambar styraciflua* at ORNL; P. t, *Pinus taeda*; P. a, *Populus alba*; P. n, *Populus nigra*; P. e, *Populus x euramericana*.

significantly lower than that of trees in control plots, in seven out of the nine years (Fig. 1a). However, when comparing $\delta^{18}O$ values recorded in tree ring of FACE plots before (1988–1997) and during (1998–2004) fumigation, we observed a significant difference ($P < 0.001$), with higher $\delta^{18}O$ during CO_2 enrichment.

At the Duke FACE, $\delta^{18}O$ varied between 26.1‰ and 30.7‰ across the entire time-period (Fig. S4b,c). The fumigation led to a slight increase in $\delta^{18}O$ of *P. taeda* during 2000–2004, while this increase was insignificant in the other years under CO_2 fumigation (Fig. 1c). In *L. styraciflua* the increase in $\delta^{18}O$ was significant ($P < 0.05$) during 1999–2003 (Fig. 1b). Moreover, when we compared the $\delta^{18}O$ values of the years before and after fumigation in FACE plots, we found small but statistically significant differences ($P < 0.05$) for both species (Fig. 1b,c).

At the POP-EUROFACE, elevated CO_2 increased the $\delta^{18}O$ of tree rings in all three genotypes and years of fumigation compared with control (Fig. 2a–c), but the difference was highly significant only in 2003 and 2004. In 2005, after the end of the fumigation, the tree ring $\delta^{18}O$ in FACE plots decreased, and no significant differences between FACE and control trees were recorded.

The relationship between $\delta^{18}O$ at elevated ($\delta^{18}O$_E) and at ambient CO_2 ($\delta^{18}O$_C) was significant across all the sites and species ($r^2 = 0.78$, $P < 0.01$; Fig. 3b). The linear regression is not significantly different from the 1:1 line, showing only a slight increase of $\delta^{18}O$ as a result of the elevated CO_2 in some species and some experimental years. The positive intercept is triggered mainly by POP-EUROFACE results and implies that $\delta^{18}O$ increases as the CO_2 response declines.

WUE$_i$ interaction with physiological and climatic parameters

The changes in ^{13}C-derived WUE$_i$ (RR_WUE$_i$) observed for the various species in the different FACE sites are well related to $\delta^{18}O$ (RR_$\delta^{18}O$) during the fumigation period, suggesting that stomatal conductance played a significant role for all the species analysed, except for *P. taeda* growing at Duke FACE, where no significant relation was found between RR_WUE$_i$ and RR_$\delta^{18}O$ (Table 3).

Comparing the elevated CO_2-induced changes in WUE$_i$ (RR_WUE$_i$) with the correspondent change in BAI values (RR_BAI) for the different species during the fumigation period, we noted a remarkable difference among species. A strong relationship was observed for the three poplar genotypes, with the maximum value for *P. x euramericana* ($r^2 = 0.91$, $P < 0.001$), a moderate but still significant relationship for the *P. taeda* and no significant relationship for *L. styraciflua*, for which the increase in WUE$_i$ appeared not to translate into enhanced tree growth (Table 3).

The relative effect of climate vs elevated CO_2 on the tree WUE$_i$ was evaluated by partial correlations. No significant differences were found between the pre-fumigation and the fumigation periods for temperature and precipitation (Notes S2). For *L. styraciflua*, the elevated CO_2 stimulation of WUE$_i$ was enhanced by high temperature and low precipitation, in the summer months, at both the ORNL and Duke FACEs (Table 4). Those results are confirmed by the high WUE$_i$ found for this species in warm and dry years such as 2002, 2003 and 2004 (Fig. 1a,b). In contrast, for *P. taeda* the elevated CO_2 stimulation of WUE$_i$ was mainly related to soil moisture, and for the three poplar genotypes it was significantly correlated with summer temperature (Table 4).

Table 3 Linear regression between intrinsic water use efficiency (WUE$_i$) vs $\delta^{18}O$ and basal area increments (BAI)

Parameter	Site	Species	$\delta^{18}O$		BAI	
			r^2	b	r^2	b
WUE$_i$	ORNL	L. s	0.65*	0.59	0.003	0.50
WUE$_i$	Duke	L. s	0.68*	0.48	0.10	0.26
WUE$_i$	Duke	P. t	0.12	0.08	0.30*	0.80
WUE$_i$	POPEUROFACE	P. a	0.45*	0.53	0.38*	0.55
WUE$_i$	POPEUROFACE	P. n	0.48*	0.35	0.74**	0.27
WUE$_i$	POPEUROFACE	P. e	0.38*	0.51	0.91***	0.70

r^2, slope coefficient (b) and the relative significant P (number of stars) from the F-test are reported. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$. L. s, *Liquidambar styraciflua*; P. t, *Pinus taeda*; P. a, *Populus alba*; P. n, *Populus nigra*; P. e, *Populus x euramericana*.

Table 4 Partial correlation between RR_WUE$_i$ and climatic factors

Controlling factor	Site	Species	r^2	Months
Precipitation	ORNL	L. s	0.64* (−)	June–July
Temperature	ORNL	L. s	0.53* (+)	June–July
Precipitation	Duke	L. s	0.68* (−)	July–August
Temperature	Duke	L. s	0.75** (+)	July–August
Soil moisture	Duke	P. t	0.86** (−)	June–July–August
Temperature	POPEUROFACE	P. a	0.70** (+)	May–June–July–August
Temperature	POPEUROFACE	P. n	0.74**(+)	May–June–July–August
Temperature	POPEUROFACE	P. e	0.69* (+)	May–June–July–August

Only the months of the year when correlations were significant are reported. The sign (+) indicates a positive correlation between parameters while the sign (−) indicates a negative correlation. RR_WUE$_i$, variation in WUE$_i$. L. s, *Liquidambar styraciflua*; P. t, *Pinus taeda*; P. a, *Populus alba*; P. n, *Populus nigra*; P. e, *Populus x euramericana*. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

Discussion

Stable isotope analysis was successfully used to confirm our first hypothesis and to clarify the mechanisms responsible for the species-specific ecophysiological responses in terms of trees growth or stomata regulation. Overall, we quantified significantly higher WUE$_i$ (inferred from $\Delta^{13}C$) in FACE plots than in controls in all species, with a reduction of stomatal conductance in *L. styraciflua* (both at ORNL and Duke FACE), and in the Poplar sp. at POP-EUROFACE. The effect of elevated CO$_2$ was more variable for *P. taeda* at Duke FACE. For all species, seasonal climate variability, mainly temperature and soil moisture, affected tree ^{13}C-derived WUE$_i$.

General Trends in $\Delta = c_i/c_a$

Previous estimates of c_i/c_a showed no significant response to elevated CO$_2$ (Ellsworth, 1999; Ainsworth & Long, 2005), leading to the conclusion that, although g_s is reduced by elevated CO$_2$, this by itself does not limit carbon uptake. Our results showed a slight but significant decrease in c_i/c_a under FACE condition for all species and sites, with the smallest decrease recorded for *P. alba* and *P. nigra* and the largest in *L. styraciflua*_DUKE (Table 2). Previous c_i/c_a values were often derived from sporadic gas exchange or isotopic measurements on single leaves and during a single growing season (Jackson *et al.*, 1994; Ellsworth, 1999), while our results integrated the physiological responses to elevated CO$_2$ of whole trees and over the entire fumigation period. Saurer *et al.* (2004) proposed three scenarios that mainly differ in the degree by which the increase in c_i follows the increase in c_a, as in the case of FACE sites: (1) c_i is kept constant thus c_i/c_a decreases and WUE$_i$ can increase strongly; (2) c_i increases proportionally to c_a and the ratio c_i/c_a is kept constant, accordingly WUE$_i$ still increases; (3) c_i increases at the same rate as c_a and the ratio c_i/c_a increases while WUE$_i$ does not change. Our study appears to correspond to the first scenario. We observed that a relatively small decrease in c_i/c_a (Table 2) translated to a significant increase in WUE$_i$ (Figs 1, 2) for all species. In particular, we observed an increase of 56% at ORNL, of 77% and 90% at Duke for *P. taeda* and *L. styraciflua*, respectively, and of 64%, 72% and 89% at POP-EUROFACE for *P. alba*, *P. nigra* and *P. x euramericana*, respectively.

Elevated CO$_2$ effects on *L. styraciflua*

A significant relationship was found for *L. styraciflua* grown at ORNL and at Duke between the FACE-induced variation in WUE$_i$ (RR_WUEi) and that in $\delta^{18}O$ (RR_$\delta^{18}O$), but there was a lack of correlation between RR_WUEi and the FACE-induced change in BAI (RR_BAI). A and g_s are strongly coupled and adjustments in both influence WUE$_i$ while $\delta^{18}O$ is thought not to be strongly influenced by A. Thus, our results suggest that the increase in WUE$_i$ of this species reduced tree water losses rather than increasing assimilation and productivity. Our results confirm previous studies (Gunderson *et al.*, 2002; Schafer *et al.*, 2002; Herrick *et al.*, 2004), where a mean relative decrease in g_s of 24% and 28% was reported for *L. styraciflua* at ORNL and Duke, respectively, throughout the study periods. Broadleaved species generally have stomata that are more responsive to elevated CO$_2$ than coniferous species (Pataki *et al.*, 1998; Ellsworth, 1999; Maier *et al.*, 2002) and *L. styraciflua* showed stomatal closure, regardless of soil moisture, and limited hydraulic modification under elevated CO$_2$ (Domec *et al.*, 2010). Thus, for this species, the high enrichment in $\delta^{18}O$ under FACE is likely caused by increased stomatal closure (Pataki *et al.*, 2003).

The high correlations between the variation of WUE$_i$ and summer temperature and precipitation (Table 4) indicate the consistent influence of climatic constraints on inter-annual variation in WUE$_i$. Air temperature is directly linked with water vapour pressure and warming may further reduce g_s under elevated CO$_2$ by affecting both assimilation and respiration. The further reduction of g_s could reduce foliar evapotranspiration, which may result in higher leaf temperature and, thereby, greater respiratory carbon loss (Korner, 2006). Previous studies conducted at the FACE sites suggested that the magnitude of the reduction in g_s for *L. styraciflua* growing under elevated CO$_2$ varied considerably with environmental factors (Warren *et al.*, 2001; Herrick *et al.*, 2004; Warren *et al.*, 2011a,b) and was most significant during the dry years, accompanied by a consistent 10–16% reduction in transpiration (Warren *et al.*, 2011a). The lack of relationship between RR_WUEi and RR_BAI (Table 3) showed that the increase in WUE$_i$ recorded for this species did not necessarily translate into increased plant growth. These results are consistent and supported by the observations that aboveground growth of *L. styraciflua* at ORNL was significantly greater under elevated CO$_2$ during the first year of treatment, diminished in the subsequent years (although enhancement of net primary productivity was sustained) and became nonstatistically different from the control in the latter years (2006–2008) (Norby *et al.*, 2010). At Duke, hardwood trees stand level growth increased in FACE compared with control plots (McCarthy *et al.*, 2010).

However, c. 90% of growth under both ambient and elevated CO_2 was contributed by pines, while *L. styraciflua*'s BAI did not significantly differed between treatments (H.R. McCarthy, unpublished data), similar to our results (r^2 between ours and McCarthy's set of BAI measurements was 0.82, $P < 0.001$). Moreover, the leaf area index (LAI) for *L. styraciflua* was relatively constant during the entire fumigation period (Schafer *et al.*, 2002; Norby *et al.*, 2003). When CO_2 enrichment does not increase foliar production and, as a consequence LAI, then a reduction in g_s can translate to a reduction of transpiration leading to stand level water savings (Warren *et al.*, 2011a), which influences the increase of $\delta^{18}O$.

Elevated CO_2 effects on *P. taeda*

For *P. taeda* our $\delta^{18}O$ results and the lack of relationship between RR_WUE$_i$ and RR_$\delta^{18}O$ confirmed the limited effects of elevated CO_2 on g_s for this species (Schafer *et al.*, 2002). Indeed, we observed a slight but significant increase in $\delta^{18}O$ in FACE plots, starting 5 yr after the onset of fumigation. This excluded that trees were accessing a different water source than control trees (Barbour, 2007), at least for the first 5 yr. Further, no significant differences were found (Notes S2) between climate data recorded at the site before and after fumigation; thus the increase in $\delta^{18}O$ can be assumed to be directly related to the CO_2 enrichment.

Possibly, ^{18}O discrimination was affected by structural changes to the tree hydraulic pathway, reported by Domec *et al.* (2009) for the fumigated trees. Structural modifications could reduce the diffusion of ^{18}O-enriched water from the sites of evaporation to the mesophyll (Pèclet effect) limiting the $\delta^{18}O$ dilution effect usually observed at the site of transpiration (Barbour *et al.*, 2000). Hence, in *P. taeda* the influence of transpiration on WUE$_i$ was limited. An important factor that could account for the observed increase in WUE$_i$ under elevated CO_2 is the increase in soil water availability attributed to increased litter accumulation (Schafer *et al.*, 2002) resulting from increased LAI (McCarthy *et al.*, 2007). Indeed, increases in LAI can result in a decrease in ^{13}C discrimination and a consistent enhancement in tree WUE$_i$ (Buchmann *et al.*, 1997). Although changes in LAI are not explicitly included in our calculation, the statistically significant relation between RR_WUE$_i$ and RR_BAI (Table 2) indicated that the FACE-induced WUE$_i$ enhancement was driven by an enhancement in assimilation (Ellsworth *et al.*, 2012). Furthermore, the positive correlation between soil moisture and RR_WUE$_i$ (Table 4) during the fumigation period, indicates that local microclimatic conditions have driven the observed increase in WUE$_i$ in *P. taeda* trees growing under elevated CO_2 rather than stomatal closure.

Elevated CO_2 effects on Poplar sp

At the POP-EUROFACE, the elevated CO_2-induced increase in WUE$_i$ for the three species was significantly related to the increase in BAI during the whole study period. Elevated CO_2 also increased LAI; the stimulation persisted after canopy closure and was followed by downregulation of photosynthesis in some cases (Calfapietra *et al.*, 2005; Liberloo *et al.*, 2005, 2006). The sustained increase in A and assimilation (Bernacchi *et al.*, 2003) is possibly associated with a reduction in g_s, as suggested by the observed changes in $\delta^{18}O$. A reduction in g_s of 19–24% across 6 yr was described by Tricker *et al.* (2005) who suggested a progression in stomatal sensitivity to the c_a increase, as also reported by Calfapietra *et al.* (2005) for *P. x euramericana*. When canopies were still open, g_s decreased, likely because of reduced stomatal density and later, when canopies gradually closed, stomatal closure trigged the response under elevated CO_2 leading to an improvement of leaf level WUE$_i$. We also found that the highest $\delta^{18}O$ values were in hot and dry years, such as 2003 and 2004, indicating that the response of g_s is also strongly dependent on climate (Kubiske *et al.*, 2006).

For all the poplar genotypes, the differences in c_i/c_a, as well as in g_s between the fumigated and control plots were reduced after the fumigation stopped, that is, in 2005. We can suggest a double mechanism involved in those changes. First, the FACE experiment might not have run long enough to reveal negative feedback of CO_2 and nutrient limitation on A, and what we observed was basically the initial stimulatory effect (Tognetti *et al.*, 2000; Korner, 2006), which does not allow us to draw conclusions about the long term trend. Second, elevated CO_2 could influence the water saving of the plants, without inducing long-term changes in anatomical traits. Yazaki *et al.* (2005) and Watanabe *et al.* (2010) analysed various conifer and broadleaved species, showing that cell and lumen diameters, vessel area and other wood structures changed differently in different species under elevated CO_2 and those alterations did not permanently affect the plants.

Conclusions

A FACE induced increase of WUE$_i$ was observed in all the five tree species investigated and was related to changes in c_a and climate. We demonstrated that the c_a rise triggered species-specific physiological responses, as well as a change in the climate sensitivity of trees. In particular, in *L. styraciflua*, warmer conditions seemed to be coupled with a reduction in transpiration, leading to higher $\delta^{13}C$ values and therefore increasing WUE$_i$ but without a parallel stimulation of tree growth. A reduction of g_s was also observed in the fast-growing *Populus* sp., accompanied by positive tree growth responses, and was partially limited by high temperature during 2003 and 2004. Finally the rise of WUE$_i$ in *P. taeda* was mainly related to soil moisture increases under elevated CO_2 and opens new questions about the ability of this isohydric species (with tight stomatal control) to withstand the expected reduction in soil water in combination with an increase in drought.

The simultaneous measurements of tree-ring cellulose isotopic variability and growth uniquely allowed us to estimate changes in tree physiology and productivity in response to elevated CO_2. The effects of elevated CO_2 on forests' hydrological balance will be highly dependent on forest species composition, and WUE$_i$ should be incorporated into conceptual frameworks for assessing the species responses to climate change.

Acknowledgements

References

Ainsworth EA, Long SP. 2005. What have we learned from 15 years of free-air CO$_2$ enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy. *New Phytologist* 165: 351–371.

Ainsworth EA, Rogers A. 2007. The response of photosynthesis and stomatal conductance to rising CO$_2$: mechanisms and environmental interactions. *Plant, Cell & Environment* 30: 258–270.

Barbour MM. 2007. Stable oxygen isotope composition of plant tissue: a review. *Functional Plant Biology* 34: 83–94.

Barbour MM, Schurr U, Henry BK, Wong SC, Farquhar GD. 2000. Variation in the oxygen isotope ratio of phloem sap sucrose from castor bean. Evidence in support of the Peclet effect. *Plant Physiology* 123: 671–679.

Barbour MM, Roden JS, Farquhar GD, Ehleringer JR. 2004. Expressing leaf water and cellulose oxygen isotope ratios as enrichment above source water reveals evidence of a Peclet effect. *Oecologia* 138: 426–435.

Battipaglia G, Jaeggi M, Saurer M, Siegwolf RTW, Cotrufo MF. 2008. Climatic sensitivity of delta O-18 in the wood and cellulose of tree rings: results from a mixed stand of *Acer pseudoplatanus* L. and *Fagus sylvatica* L. *Palaeogeography Palaeoclimatology Palaeoecology* 261: 193–202.

Bernacchi CJ, Calfapietra C, Davey PA, Wittig VE, Scarascia-Mugnozza GE, Raines CA, Long SP. 2003. Photosynthesis and stomatal conductance responses of poplars to free-air CO$_2$ enrichment (PopFACE) during the first growth cycle and immediately following coppice. *New Phytologist* 159: 609–621.

Biondi F. 1999. Comparing tree-ring chronologies and repeated timber inventories as forest monitoring tools. *Ecological Applications* 9: 216–227.

Boettger T, Haupt M, Knoller K, Weise SM, Waterhouse JS, Rinne KT, Loader NJ, Sonninen E, Jungner H, Masson-Delmotte V *et al.* 2007. Wood cellulose preparation methods and mass spectrometric analyses of delta C-13, delta O-18, and nonexchangeable delta H-2 values in cellulose, sugar, and starch: an interlaboratory comparison. *Analytical Chemistry* 79: 4603–4612.

Buchmann N, Kao WY, Ehleringer J. 1997. Influence of stand structure on carbon-13 of vegetation, soils, and canopy air within deciduous and evergreen forests in Utah, United States. *Oecologia* 110: 109–119.

Calfapietra C, Tulva I, Eensalu E, Perez M, De Angelis P, Scarascia-Mugnozza G, Kull O. 2005. Canopy profiles of photosynthetic parameters under elevated CO$_2$ and N fertilization in a poplar plantation. *Environmental Pollution* 137: 525–535.

Cotrufo MF, Ineson P, Scott A. 1998. Elevated CO$_2$ reduces the nitrogen concentration of plant tissues. *Global Change Biology* 4: 43–54.

Domec JC, Palmroth S, Ward E, Maier CA, Therezien M, Oren R. 2009. Acclimation of leaf hydraulic conductance and stomatal conductance of *Pinus taeda* (loblolly pine) to long-term growth in elevated CO$_2$ (free-air CO$_2$ enrichment) and N-fertilization. *Plant, Cell & Environment* 32: 1500–1512.

Domec JC, Schafer K, Oren R, Kim HS, McCarthy HR. 2010. Variable conductivity and embolism in roots and branches of four contrasting tree species and their impacts on whole-plant hydraulic performance under future atmospheric CO$_2$ concentration. *Tree Physiology* 30: 1001–1015.

Ellsworth DS. 1999. CO$_2$ enrichment in a maturing pine forest: are CO$_2$ exchange and water status in the canopy affected? *Plant, Cell & Environment* 22: 461–472.

Ellsworth DS, Thomas R, Crous KY, Palmroth S, Ward E, Maier C, Delucia E, Oren R. 2012. Elevated CO$_2$ affects photosynthetic responses in canopy pine and subcanopy deciduous trees over 10 years: a synthesis from Duke FACE. *Global Change Biology* 18: 223–242.

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, Lloyd J, Taylor JA, Flanagan LB, Syvertsen JP, Hubick KT, Wong SC, Ehleringer JR. 1993. Vegetation effects on the isotope composition of oxygen in atmospheric CO$_2$. *Nature* 363: 439–443.

Farquhar GD, O'Leary MH, Berry JA. 1982. On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Australian Journal of Plant Physiology* 9: 121–137.

Field CB, Jackson RB, Mooney HA. 1995. Stomatal responses to increased CO$_2$ - implications from the plant to the global-scale. *Plant, Cell & Environment* 18: 1214–1225.

Fritts HC. 1976. *Tree rings and climate.* London, UK; New York, NY, San Francisco, USA: Academic Press.

Gagen M, Finsinger W, Wagner-Cremer F, McCarroll D, Loader NJ, Robertson I, Jalkanen R, Young G, Kirchhefer A. 2011. Evidence of changing intrinsic water-use efficiency under rising atmospheric CO(2) concentrations in Boreal Fennoscandia from subfossil leaves and tree ring delta 13C ratios. *Global Change Biology* 17: 1064–1072.

Gielen B, Calfapietra C, Lukac M, Wittig VE, De Angelis P, Janssens IA, Moscatelli MC, Grego S, Cotrufo MF, Godbold DL *et al.* 2005. Net carbon storage in a poplar plantation (POPFACE) after three years of free-air CO$_2$ enrichment. *Tree Physiology* 25: 1399–1408.

Gunderson CA, Sholtis JD, Wullschleger SD, Tissue DT, Hanson PJ, Norby RJ. 2002. Environmental and stomatal control of photosynthetic enhancement in the canopy of a sweetgum (*Liquidambar styraciflua* L.) plantation during 3 years of CO$_2$ enrichment. *Plant, Cell & Environment* 25: 379–393.

Gunderson CA, Wullschleger SD. 1994. Photosynthetic acclimation in trees to rising atmospheric CO$_2$ - a broader perspective. *Photosynthesis Research* 39: 369–388.

Hedges LV, Gurevitch J, Curtis PS. 1999. The meta-analysis of response ratios in experimental ecology. *Ecology* 80: 1150–1156.

Hendrey GH, Ellsworth DS, Lewin KF, Nagy J. 1999. A free-air enrichment system for exposing tall forest vegetation to elevated atmospheric CO$_2$. *Global Change Biology* 5: 293–309.

Herrick JD, Maherali H, Thomas RB. 2004. Reduced stomatal conductance in sweetgum (*liquidambar styraciflua*) sustained over long-term CO$_2$ enrichment. *New Phytologist* 162: 387–396.

Hill SA, Waterhouse JS, Field EM, Switsur VR, AP Rees T. 1995. Rapid recycling of triose phosphates in oak stem tissue. *Plant, Cell & Environment* 18: 931–936.

Jackson RB, Sala OE, Field CB, Mooney HA. 1994. CO$_2$ alters water-use, carbon gain, and yield for the dominant species in a natural grassland. *Oecologia* 98: 257–262.

Keel SG, Siegwolf RTW, Korner C. 2006. Canopy CO$_2$ enrichment permits tracing the fate of recently assimilated carbon in a mature deciduous forest. *New Phytologist* 172: 319–329.

Kleinbaum DG, Kupper LL, Muller KE, Nizam A. 2008. *Applied regression analysis and multivariable methods.* Belmont, CA, USA: Thomson Higher Education.

Korner C. 2006. Plant CO$_2$ responses: an issue of definition, time and resource supply. *New Phytologist* 172: 393–411.

Kubiske ME, Quinn VS, Heilman WE, McDonald EP, Marquardt PE, Teclaw RM, Friend AL, Karnosky DF. 2006. Interannual climatic variation mediates elevated CO$_2$ and O-3 effects on forest growth. *Global Change Biology* 12: 1054–1068.

Leakey ADB, Ainsworth EA, Bernacchi CJ, Rogers A, Long SP, Ort DR. 2009. Elevated CO_2 effects on plant carbon, nitrogen, and water water relations: six important lessons from face. *Journal of Experimental Botany* **60**: 2859–2876.

Liberloo M, Calfapietra C, Lukac M, Godbold D, Luos ZB, Polle A, Hoosbeek MR, Kull O, Marek M, Raines C *et al.* 2006. Woody biomass production during the second rotation of a bio-energy populus plantation increases in a future high CO_2 world. *Global Change Biology* **12**: 1094–1106.

Liberloo M, Dillen SY, Calfapietra C, Marinari S, Bin Luo Z, De Angelis P, Ceulemans R. 2005. Elevated CO_2 concentration, fertilization and their interaction: growth stimulation in a short-rotation poplar coppice (EUROFACE). *Tree Physiology* **25**: 179–189.

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 Series B – Chemical and Physical Meteorology* **43**: 322–330.

Maier CA, Johnsen KH, Butnor J, Kress LW, Anderson PH. 2002. Branch growth and gas exchange in 13-year-old loblolly pine (*Pinus taeda*) trees in response to elevated carbon dioxide concentration and fertilization. *Tree Physiology* **22**: 1093–1106.

McCarroll D, Loader NJ. 2004. Stable isotopes in tree rings. *Quaternary Science Reviews* **23**: 771–801.

McCarthy HR, Oren R, Finzi AC, Ellsworth DS, Kim HS, Johnsen KH, Millar B. 2007. Temporal dynamics and spatial variability in the enhancement of canopy leaf area under elevated atmospheric CO_2. *Global Change Biology* **13**: 2479–2497.

McCarthy HR, Oren R, Johnsen KH, Gallet-Budynek A, Pritchard SG, Cook CW, LaDeau SL, Jackson RB, Finzi AC. 2010. Reassessment of plant carbon dynamics at the Duke free-air CO_2 enrichment site: interactions of atmospheric CO_2 with nitrogen and water availability over stand development. *New Phytologist* **185**: 514–528.

Miglietta F, Peressotti A, Vaccari FP, Zaldei A, deAngelis P, Scarascia-Mugnozza G. 2001. Free-air CO_2 enrichment (FACE) of a poplar plantation: the POPFACE fumigation system. *New Phytologist* **150**: 465–476.

Monserud RA, Marshall JD. 2001. Time-series analysis of delta C-13 from tree rings. I. *Time trends and autocorrelation. Tree Physiology* **21**: 1087–1102.

Norby RJ, Sholtis JD, Gunderson CA, Jawdy SS. 2003. Leaf dynamics of a deciduous forest canopy: no response to elevated CO_2. *Oecologia* **136**: 574–584.

Norby RJ, Todd DE, Fults J, Johnson DW. 2001. Allometric determination of tree growth in a CO_2-enriched sweetgum stand. *New Phytologist* **150**: 477–487.

Norby RJ, Warren JM, Iversen CM, Medlyn BE, McMurtrie RE. 2010. Co(2) enhancement of forest productivity constrained by limited nitrogen availability. *Proceedings of the National Academy of Sciences, USA* **107**: 19368–19373.

Norby RJ, Zak DR. 2011 Ecological lessons from free-air CO_2 enrichment (face) experiments. In: Futuyma DJ, Shaffer HB, Simberloff D, eds. *Annual review of ecology, evolution, and systematics*, vol 42. Palo Alto, CA, USA: Annual Reviews, 181–203.

Pataki DE, Ellsworth DS, Evans RD, Gonzalez-Meler M, King J, Leavitt SW, Lin GH, Matamala R, Pendall E, Siegwolf R *et al.* 2003. Tracing changes in ecosystem function under elevated carbon dioxide conditions. *BioScience* **53**: 805–818.

Pataki DE, Oren R, Tissue DT. 1998. Elevated carbon dioxide does not affect average canopy stomatal conductance of *Pinus taeda* L. *Oecologia* **117**: 47–52.

Penuelas J, Canadell JG, Ogaya R. 2011. Increased water-use efficiency during the 20th century did not translate into enhanced tree growth. *Global Ecology and Biogeography* **20**: 597–608.

Picon C, Ferhi A, Guehl JM. 1997. Concentration and delta C-13 of leaf carbohydrates in relation to gas exchange in quercus robur under elevated CO_2 and drought. *Journal of Experimental Botany* **48**: 1547–1556.

Riggs JS, Tharp M, Norby R. 2003. ORNL FACE weather data [WWW document] URL http://cdiac.ornl.gov.programs/FACE/ornldata/weatherfiles.html Carbon dioxide Information Analysis Center, Oak Ridge, Tenn [accessed 2 June 2005].

Roden JS, Ehleringer JR. 2000. Hydrogen and oxygen isotope ratios of tree ring cellulose for field-grown riparian trees. *Oecologia* **123**: 481–489.

Saurer M, Siegwolf RTW, Schweingruber FH. 2004. Carbon isotope discrimination indicates improving water-use efficiency of trees in northern Eurasia over the last 100 years. *Global Change Biology* **10**: 2109–2120.

Schafer KVR, Oren R, Lai CT, Katul GG. 2002. Hydrologic balance in an intact temperate forest ecosystem under ambient and elevated atmospheric CO_2 concentration. *Global Change Biology* **8**: 895–911.

Scheidegger Y, Saurer M, Bahn M, Siegwolf R. 2000. Linking stable oxygen and carbon isotopes with stomatal conductance and photosynthetic capacity: a conceptual model. *Oecologia* **125**: 350–357.

Schweingruber FH. 1988. *Tree rings: basics and applications of dendrochronology*. Dordrecht, the Netherlands: Kluwer Academic Publishers.

Schweingruber FH. 1996. Dendrochronology – an extremely exact measuring tool for the study of environmental and human history. *Naturwissenschaften* **83**: 370–377.

Seibt U, Rajabi A, Griffiths H, Berry JA. 2008. Carbon isotopes and water use efficiency: sense and sensitivity. *Oecologia* **155**: 441–454.

Sholtis JD, Gunderson CA, Norby RJ, Tissue DT. 2004. Persistent stimulation of photosynthesis by elevated CO_2 in a sweetgum (Liquidambar styraciflua) forest stand. *New Phytologist* **162**: 343–354.

Springer CJ, Thomas RB. 2007. Photosynthetic responses of forest understory tree species to long-term exposure to elevated carbon dioxide concentration at the Duke Forest FACE experiment. *Tree Physiology* **27**: 25–32.

Tognetti R, Cherubini P, Innes JL. 2000. Comparative stem-growth rates of Mediterranean trees under background and naturally enhanced ambient CO_2 concentrations. *New Phytologist* **146**: 59–74.

Tricker PJ, Trewin H, Kull O, Clarkson GJJ, Eensalu E, Tallis MJ, Colella A, Doncaster CP, Sabatti M, Taylor G. 2005. Stomatal conductance and not stomatal density determines the long-term reduction in leaf transpiration of poplar in elevated CO_2. *Oecologia* **143**: 652–660.

Warren CR, McGrath JF, Adams MA. 2001. Water availability and carbon isotope discrimination in conifers. *Oecologia* **127**: 476–486.

Warren JM, Norby RJ, Wullschleger SD. 2011a. Elevated CO_2 enhances leaf senescence during extreme drought in a temperate forest. *Tree Physiology* **31**: 117–130.

Warren JM, Poetzelsberger E, Wullschleger SD, Thornton PE, Hasenauer H, Norby RJ. 2011b. Ecohydrologic impact of reduced stomatal conductance in forests exposed to elevated CO_2. *Ecohydrology* **4**: 196–210.

Watanabe Y, Satomura T, Sasa K, Funada R, Koike T. 2010. Differential anatomical responses to elevated CO_2 in saplings of four hardwood species. *Plant, Cell & Environment* **33**: 1101–1111.

Wullschleger SD, Tschaplinski TJ, Norby RJ. 2002. Plant water relations at elevated CO_2 – implications for water-limited environments. *Plant, Cell & Environment* **25**: 319–331.

Yazaki K, Muruyama Y, Mori S, Koibe T, Funada R. 2005 Effect of elevated carbon dioxide concentration on wood structure and formation in trees. In: Omasa K, Nouchi I, De Kok LD, eds. *Plant responses to air pollution and global change*. Tokyo, Japan: Sprinter-Verlag, 89–97.

Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Mean annual temperature and anomalies, mean annual precipitation and anomalies related to ORNL.

Fig. S2 Mean annual temperature and anomalies, mean annual precipitation and anomalies related to Duke.

Fig. S3 Mean annual temperature and anomalies, mean annual precipitation and anomalies related to POP-EUROFACE.

Fig. S4 Average chronologies of BAI, $\delta^{18}O$ and Δ chronologies for *Liquidambar styraciflua* at ORNL and Duke and for *Pinus taeda* at Duke.

Fig. S5 Average chronologies of BAI, $\delta^{18}O$ and Δ chronologies for *Populus alba*, *Populus nigra* and *Populus x. euramericana*.

Notes S1 Description of the three FACE sites and of the atmospheric CO_2 enrichment supplies

Notes S2 Description of the meteorological data used to calculate annual temperature and precipitation averages and the corresponding anomalies

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.

About *New Phytologist*

- *New Phytologist* is an electronic (online-only) journal owned by the New Phytologist Trust, a **not-for-profit organization** dedicated to the promotion of plant science, facilitating projects from symposia to free access for our Tansley reviews.

- Regular papers, Letters, Research reviews, Rapid reports and both Modelling/Theory and Methods papers are encouraged. We are committed to rapid processing, from online submission through to publication 'as ready' via *Early View* – our average time to decision is <25 days. There are **no page or colour charges** and a PDF version will be provided for each article.

- The journal is available online at Wiley Online Library. Visit **www.newphytologist.com** to search the articles and register for table of contents email alerts.

- If you have any questions, do get in touch with Central Office (np-centraloffice@lancaster.ac.uk) or, if it is more convenient, our USA Office (np-usaoffice@ornl.gov)

- For submission instructions, subscription and all the latest information visit **www.newphytologist.com**