Increased water-use efficiency during the 20th century did not translate into enhanced tree growth

Josep Peñuelas¹*, Josep G Canadell² and Romà Ogaya¹

Aims The goals of this study are: (1) to determine whether increasing atmospheric CO₂ concentrations and changing climate increased intrinsic water use efficiency (iWUE, as detected by changes in Δ¹³C) over the last four decades; and if it did increase iWUE, whether it led to increased tree growth (as measured by tree-ring growth); (2) to assess whether CO₂ responses are biome dependent due to different environmental conditions, including availability of nutrients and water; and (3) to discuss how the findings of this study can better inform assumptions of CO₂ fertilization and climate change effects in biospheric and climate models.

Methods The analysis encompassed 47 study sites including boreal, wet temperate, mediterranean, semi-arid and tropical biomes for which measurements of tree ring Δ¹³C and growth are available over multiple decades.

Results The iWUE inferred from the Δ¹³C analyses of comparable mature trees increased 20.5% over the last 40 years with no significant differences between biomes. This increase in iWUE did not translate into a significant overall increase in tree growth. Half of the sites showed a positive trend in growth while the other half had a negative or no trend. There were no significant trends within biomes or among biomes.

Main conclusions These results show that despite an increase in atmospheric CO₂ concentrations of over 50 p.p.m. and a 20.5% increase in iWUE during the last 40 years, tree growth has not increased as expected, suggesting that other factors have overridden the potential growth benefits of a CO₂-rich world in many sites. Such factors could include climate change (particularly drought), nutrient limitation and/or physiological long-term acclimation to elevated CO₂. Hence, the rate of biomass carbon sequestration in tropical, arid, mediterranean, wet temperate and boreal ecosystems may not increase with increasing atmospheric CO₂ concentrations as is often implied by biospheric models and short-term elevated CO₂ experiments.

Keywords Climate change, CO₂ fertilization, Δ¹³C, drought, forest, geographical range, tree growth, water use efficiency.
Overdieck & Forstreuter, 1994; Picon et al., 1996; Morgan et al., 2004). An analysis across multiple sites suggests that the growth response of trees to elevated CO₂ is relatively predictable across a broad range of sites, with an average increase of 23% at a median CO₂ of double the pre-industrial level (Norby et al., 2005). This increased net primary productivity (NPP) in response to increasing atmospheric CO₂ is what is believed to be the dominant driver of the growing terrestrial carbon sink in the recent past and over the next decades as modelled by the state-of-the-art Earth system models (Friedlingstein et al., 2006; Sitch et al., 2008). Under these conditions, the terrestrial sink is expected to remove well above 150 Pg C of anthropogenic emissions over the next 50 years, significantly slowing down climate change. Failure to model a strong CO₂ fertilization effect results in the switch of the terrestrial biosphere from a CO₂ sink into a net CO₂ source because of enhanced heterotrophic respiration under the concomitant effect of warming (Sitch et al., 2008). Of concern is the fact that the NPP response to raising CO₂ is the single most uncertain process in predicting the magnitude of the future carbon–climate feedback (Denman et al., 2007) and therefore the fraction of anthropogenic emissions that will accumulate in the atmosphere causing climate change (Canadell et al., 2007a; Le Quéré et al., 2009).

At present, our state of knowledge about the long-term effects of CO₂ on NPP is largely underpinned by experimental evidence collected from CO₂ enrichment experiments and paleoecorecords for which little process attribution is possible. It is acknowledged that there are shortcomings to both approaches and that other complementary techniques are necessary to further constrain the effects of increasing CO₂ on biomass accumulation. For example, most experimental studies have been conducted during short periods of time (relative to the plant’s life cycle) in growth chambers or free air CO₂-enrichment (FACE) systems with seedlings or young growing plants. That is unlike the conditions experienced by mature forests growing in a natural environment with a steady-state nutrient cycle and subject to other factors that influence plant growth and therefore the response of NPP to elevated CO₂ concentrations (e.g. Karnosky, 2003; Luo et al., 2004; Norby et al., 2009). Likewise, short experiments (several years) do not make it possible for plants to adjust their physiological response over time to gradually increasing CO₂ concentrations (Long et al., 2004; Domec et al., 2009).

Results from experiments with fully coupled systems in which the natural nutrient cycling governs growth at steady-state leaf area index (LAI) yield rather moderate or no effects of elevated CO₂ on plant biomass production (Oren et al., 2001; Shaw et al., 2002; Körner, 2006; Norby et al., 2009). Experiments with multiple CO₂ × climate/environment interactions (water, temperature, nutrient supply) yield no overall CO₂ effect in grassland systems (Shaw et al., 2002), highlighting the significant influence of co-limiting environmental factors. In fact, it has been suggested that greatest CO₂ fertilization effects may be seen in savanna systems post-fire (Bond & Midgley, 2000; Bond et al., 2003; Hungate et al., 2006), where nutrients are less limiting because of the nutrient input from ashes. From these experiments, at least four main constraints have been observed to limit the CO₂ fertilization effect: (1) element stoichiometry (nutrient imbalance through deficiency of one or more nutrients, often nitrogen and phosphorus); (2) forest tree dynamics (mature trees and closed canopy system are less responsive); (3) possible acclimation to gradually increasing CO₂ concentrations; and (4) secondary effects of CO₂ on water relations and biodiversity (often leading to structural changes less favourable for response to elevated CO₂). Given our limited knowledge of these effects globally, the magnitude of the terrestrial CO₂ fertilization effect remains uncertain.

In this paper, we explore more integrated and long-term records of the effects of elevated CO₂ on the intrinsic water use efficiency (iWUE) and tree growth through the study of tree rings over multiple decades. Variation in iWUE estimated as the ratio between photosynthesis and stomatal conductance is recorded in the variation of the carbon isotope discrimination Δ¹³C of the annual growth rings that are laid down during each growing season (e.g. Duquesnay et al., 1998). Long-term changes in the gas exchange metabolism of established trees will be recorded in the variation of this carbon isotope discrimination Δ¹³C, which will give insight into how naturally growing trees respond, or have they responded, to increasing atmospheric CO₂ concentrations. Several studies using Δ¹³C in trees growing in natural conditions all over the world (see Table 1 and Fig. 1 for sites and references) show that trees vary in their responses to the increasing atmospheric CO₂ concentrations in an interactive way with other changing environmental factors. The apparent reason is that the carbon isotope discrimination Δ¹³C in tree rings also responds strongly to many other environmental factors, especially climatic ones such as growing season temperature, relative humidity and precipitation (Switsur & Waterhouse, 1998, and references therein). Climate has changed in the last decades towards warmer conditions (IPCC, 2007). These warmer conditions have strongly affected plant function (Peñuelas & Filella, 2001; Peñuelas & Boada, 2003; Fischlin et al., 2007) and so long-term changes in the gas exchange metabolism of established trees are expected.

Other studies on naturally growing trees have focused on growth rates using tree rings to estimate tree growth over time and its response to environmental changes including increasing atmospheric CO₂ concentration and climate change (see sites and references in Table 1 and Fig. 1). Often these studies show that trees did not significantly increase growth in recent decades despite increasing atmospheric CO₂ concentration. In some cases, trees showed a decline in growth related to warming and drought, for example, at the southernmost distribution edge of temperate trees (Jump et al., 2006, 2007; Peñuelas et al., 2007, 2008; Piovesan et al., 2008; and references and sites depicted in Table 1) and there is even a potential for amplified tree mortality due to drought and heat in forests world-wide (Allen et al., 2010).

In this paper we report a survey and meta-analysis of published studies on carbon isotope composition and growth of tree rings to investigate iWUE and growth changes during a 40-year period of rapid atmospheric and climate change (from the 1960s
<table>
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to the 2000s). The analysis includes forests in tropical, arid, mediterranean, wet temperate and boreal regions. The overall goal of this study is to determine whether there was a discernible CO₂ fertilization effect in rates of tree growth during the period from the 1960s to the 2000s as a likely result of increasing atmospheric CO₂ concentrations, or, on the contrary, whether other environmental factors override such an effect. The specific goals are: (1) to determine whether increasing atmospheric CO₂ concentrations and changing climate increased iWUE (as detected by changes in Δ₁³C), and whether that led to increased tree growth (as measured by tree-ring growth); (2) to assess whether CO₂ responses are biome dependent due to different environmental conditions including availability of nutrients and water; and (3) to discuss how the findings of this study can better inform assumptions of CO₂ fertilization effects in biospheric and climate models.

MATERIALS AND METHODS

Study sites, sampling trees and analyses

We analysed tree ring isotopic and growth data from 47 sites for which either isotopic data or growth data or both were available in the published literature. The 47 sites included tropical, arid, mediterranean, wet temperate and boreal forests distributed throughout Europe, Asia, Africa, America and Oceania (Table 1, Fig. 1). The bulk of the study sites were not disturbed sites, and the very few disturbed ones did not present any differential response, except for site number 30 (see Table 1) after a strong seismic disturbance.

Only data from control treatments were used in studies where fertilization or other treatments were applied. Preparation and analyses of tree cores is described in the surveyed studies (references in Table 1).

Δ₁³C and calculation of water use efficiency

Δ₁³C (carbon isotopic discrimination) was calculated as: 
\[ \Delta_{13}C = \frac{\delta_{air} - \delta_{plant}}{1000 - \delta_{plant}} \times 1000 \]
following Farquhar et al. (1982) and using published values for air Δ₁³C from ice core measurements, direct atmospheric measurements and inferred from C₄ plants (McCarroll & Loader, 2004). Δ₁³C is related to Cᵢ (intercellular CO₂ concentration) and Cₐ (ambient CO₂ concentration) by the following equation:

\[ \Delta_{13}C_{10} = a + (b - a)(C_i / C_a) \]  

(1)

where \( a \) is the discrimination against ¹³CO₂ during diffusion of CO₂ through stomata (\( a = 4.4 \text{‰}; \) O’Leary, 1981), \( b \) is the discrimination associated with carboxylation (\( b = 27 \text{‰}; \) Farquhar & Richards, 1984).

Fick’s law is:

\[ A = g_{CO₂}(C_a - C_i) \]  

(2)

where \( A \) is the net photosynthesis, measured as CO₂ uptake, and \( g_{CO₂} \) is the leaf conductance to CO₂. The leaf conductance to water vapour, \( g_{H₂O} \), is related to \( g_{CO₂} \) by:

\[ g_{H₂O} = 1.6g_{CO₂} \]  

(3)

Given equations (1), (2) and (3), Δ₁³C can be related to the ratio \( A / g_{H₂O} \) (iWUE) (Osmond et al., 1980) by the following equation:

Figure 1  Map of the location of the sites studied (Mollweide projection, central meridian 0°).
Standardized tree ring width measurements

Ring width in trees changes with age. Thus, if a change in growth trend is suspected, it may be impossible to investigate it on the basis of changes in ring width alone (Phipps & Whiton, 1988). In a few studies, the conversion of radial increment (ring width) into basal area increment (BAI) overcomes this problem (Phipps & Whiton, 1988; Pedersen, 1998; see details in Jump et al., 2006). We focused only on the variation between levels of growth during the mature phase of growth, marked by the abrupt change in slope (levelling off) after the release phase. We did not consider the data for the first stage in the life of a tree before reaching maturity. In most other studies, the individual tree ring width (TRW) series were standardized to remove non-climatic, tree-geometry and/or age-related trends (Cook & Kairiukstis, 1990). Standardized TRW indices for each tree were calculated as $I = R/G$, where $I$ is the value of the index, $R$ the measured ring width and $G$ is the modelled ring width based on the best-fit exponential growth trend.

Data treatment and statistical analysis

Latitude, longitude, altitude and distance to sea (continentality) were recorded for each studied site. Regression analyses were used to calculate the slope (annual change) in the temporal trends of $\Delta^{13}C$, iWUE and standardized TRW of mature trees in each study over the last 40 years. A meta-analysis comparing $\Delta^{13}C$, iWUE and growth in the 1960s and 2000s was conducted using Metawin 2.0 (Sinauer Associates, Inc., Sunderland, MA, USA) with the data for which standard deviation (SD) of the mean was provided (only 11 sites). General linear models with $\Delta^{13}C$, iWUE and growth as dependent variables and biome, distance to the sea, altitude, latitude and forest age as explanatory variables were conducted for all sites with available data. Generalized linear models (binomial family) were also constructed with growth (increased or not) as the dependent binomial variable and the same independent variables as explanatory variables. Dependent t-test comparisons between growth, $\Delta^{13}C$ and iWUE in the 1960s and 2000s were conducted, as well as ANOVA analyses by biomes. All these statistical calculations and analyses were conducted using the Statistica software package (StatSoft, Inc. Tulsa, OK, USA).

RESULTS

The meta-analyses conducted with the results from the 12 sites for which SD was provided showed no significant change between the 1960s and 2000s in $\Delta^{13}C$ and growth rates, both for the entire dataset and by biome. However, iWUE, significantly increased by 20% (data not shown).

When using all sites in the meta-analyses (both with and without SD), the results were similar with no significant change in the isotopic discrimination ($\Delta^{13}C$) between the 1960s and 2000s. $\Delta^{13}C$ remained at c. 16‰ throughout the 40-year period (Fig. 2). The iWUE significantly increased from 90 to c. 109 mmol mol$^{-1}$, i.e. 20.5% (Fig. 2). The increase in growth for the overall set of studies was not statistically significant (Fig. 2). There was an increase in growth in 18 of the 35 studies with growth data. Another 6 sites had similar growth and the other 11 sites had smaller growth in the 2000s than in the 1960s. Thus the estimated iWUE of mature trees increased with atmospheric CO$_2$ concentrations but it did not translate into significantly more overall growth (Fig. 2).

No significant differences were found in any of the three variables studied ($\Delta^{13}C$, iWUE and growth) among the five biomes studied (tropical, arid, mediterranean, wet temperate and boreal; Fig. 3) nor in the relationships with latitude, altitude, tree age or continentality (data not shown). There was a mediterranean site with a very large increase of growth from 1960 to 2000 (137.9%, Table 1 study number 30) that was not used for the calculation of the mean growth in mediterranean sites as Abies cephalonica is more of a wet mountain tree and the site experienced the influence of a strong seismic disturbance; however, even if we considered that species and site, the differences from the other biomes were still not significant.

Figure 2 Mean overall values of $\Delta^{13}C$, intrinsic water use efficiency (iWUE) and growth (standardized tree ring width, TRW) of mature trees in the early 1960s and in the early 2000s. Three asterisks indicate significantly different values between sites ($P < 0.001$; dependent t-test analysis), and NS indicates no significant difference in values between sites. Error bars indicate SE.
DISCUSSION

The iWUE increased by an average of 20.5% over the second half of the 20th century as expected from the fast build-up of atmospheric CO2. The magnitude of change is in agreement with previous reports that found an increase of iWUE during the same period by using historical records in both leaves and tree rings (Peñuelas & Matamala, 1990; Peñuelas & Azcón-Bieto, 1992; Woodward, 1993; Ehleringer & Cerling, 1995; Feng & Epstein, 1995; Bert et al., 1997; Duquesnay et al., 1998; Feng, 1999; Peñuelas et al., 2008). This increase in iWUE is linked to the sharp increase in internal CO2 concentration because of the rise of atmospheric CO2 concentration. However, despite higher iWUE, the expected increase in photosynthetic rates in response to rising atmospheric CO2 (Long et al., 2004) and the warming-induced lengthening of the growing season for many species (Peñuelas & Filella, 2001; Peñuelas et al., 2002), there was no overall significant increase of plant growth over the study period. Thus, the CO2 fertilization effect was either not realized or was not big enough to overcome the concomitant negative effects of climate change, e.g. drought, nutrient scarcity or photosynthetic acclimation to elevated CO2 on plant growth.

An important part of the increase in iWUE results from the effect of the relative increase in Ca over the past 40 years (50 p.p.m./335 p.p.m.) which should increase iWUE by c. 15%, i.e. only a little bit less than the 20.5% average increase found here. Thus C/Ca would not have changed much over 40 years in trees as a whole. A near constancy of C/Ca implies that if stomatal conductance decreases, then so does leaf photosynthetic capacity. The small additional increment in iWUE can result from increasing net photosynthesis (A), decreasing leaf conductance (gco), or a combination of these factors. It is even possible that a decrease in gco in response to rising atmospheric CO2 concentration or/and increasing temperatures, and hence water stress, could cause the increase in iWUE with no associated change, or even a decline, in A, although smaller than the decrease in gco. Plants growing in CO2-enriched atmospheres with suboptimal resources acclimate to the greater CO2 availability by lowering photosynthetic capacity below the expected for a given CO2 concentration (Long et al., 2004). While instantaneous CO2 responses can be well described by the photosynthesis model of Farquhar et al. (1980), photosynthetic acclimation to elevated CO2 is much more complex to simulate and requires consideration of additional feedbacks such as water or nutrient availability (Gutschick, 2007, for a review). Increasing temperatures could also produce higher respiration rates, which would lower NPP and thus TRW and BAI, and have a negative impact on the assimilation capacity of the leaves. Most studies on trees indicate, however, that their respiration substantially acclimates to temperature changes (Atkin et al., 2005), which would not explain the iWUE/growth discrepancy.

Regardless of the ultimate causation of the observed response, the data show that an increase in iWUE does not necessarily translate into increased plant growth. Thus, we show that a number of forests in diverse biomes have already saturated in response to further increases in atmospheric CO2 given other concurrent growth-limiting factors. This finding is consistent and supported by many experimental studies (see reviews by Körner et al., 2007, and Oren et al., 2001; see also Canadell et al., 2007b; Norby et al., 2009). Nutrient limitations (mostly N and P) prevent the full expression of the CO2 fertilization effect on plant growth and seem to drive the saturation of the plant CO2 response (Luo et al., 2004; Edwards et al., 2005; Reich et al., 2006; Norby et al., 2009), and may thus explain the lack of growth responses to increased CO2 at some of the sites.

The increased iWUE often results in a slower rate of depletion of soil moisture, favouring plants in arid ecosystems. CO2-induced changes in soil moisture are suggested to account for a significant component of the CO2 fertilization effect such as that found in woody plants in a steppe grassland (Morgan et al., 2007). However, in our study we found no significant differences in responses between arid and wet ecosystems. Other factors limiting plant growth, such as nutrient limitations or acclimation, may have precluded such responses.

Whichever mechanism or mechanisms underlying this response, the lack of expected growth does not support some of the assumptions and model outputs which predict a continuing rise in terrestrial carbon storage as a result of a strong CO2 effect alone over the next half a century (Melillo et al., 1996; Cao & Woodward, 1998; Friedlingstein et al., 2006; Leuzinger et al., 2001; see also Canadell et al., 2007b; Norby et al., 2009).
Körner, 2007; Sitch et al., 2008). This lack of growth does not support either the expected growth response following the increase of biologically reactive nitrogen in the atmosphere over the past century, and especially in recent decades as a consequence of the use of fossil fuels and fertilizers (Magnani et al., 2007; Janssens et al., 2010), and is not easily reconciled with results reporting recent biomass accumulation greatly exceeded the expected growth caused by natural recovery from disturbance (McMahon et al., 2010). These uncertain and apparently contradictory results warrant further study to get a better understanding of the changes in growth rates in forest systems, which influence current and future states of the atmosphere and biosphere.

If the expectation of a significant CO₂ fertilization effect is not realized, future terrestrial carbon storage will be overestimated, leading to faster accumulation of atmospheric CO₂ and associated climate change. It is thus critical that models are able to reproduce past long-term trends in ecosystem dynamics in addition to gain knowledge from short-term experiments.

Lack of a detectable net effect of the CO₂ fertilization effect on tree growth in our analysis does not negate the existence of a terrestrial net carbon sink and its possible growth in the future. In fact, there are now multiple lines of independent evidence that confirm the existence of a terrestrial net sink and its growth over the last decades (Canadell et al., 2007a; Le Quéré et al., 2009). Our findings suggest that the drivers of the net extratropical terrestrial sink (largely in the Northern Hemisphere) may be dominated by processes other than the CO₂ fertilization effect such as forest regrowth after land abandonment, woody encroachment and thickening. A key consequence of this set of drivers is that they are the legacy of past land-use changes and that their effects on carbon sequestration will diminish as forest regrowth reaches maturity and no additional land is abandoned. Our results also point out the lack of knowledge of the CO₂ fertilization effect in tropical systems thought to play an equal or even larger role in terrestrial CO₂ uptake (Stephens et al., 2007). To date, no elevated CO₂ experiments have been undertaken in tropical forest ecosystems.

CONCLUSIONS

Changes in carbon isotope composition of tree rings show increasing iWUE in the last decades, implying that the ratio between assimilation rates and stomatal conductance has increased. The results emphasize that the global rise of atmospheric CO₂ and changing climate are already influencing the gas exchange of forests and might be decreasing plant water use. However, an increase in iWUE alone is not sufficient to produce increased plant growth. Other factors such as increasing temperature, drought, nutrient limitation and/or plant acclimation may preclude such growth increase. The lack of an increase in tree growth with increasing atmospheric CO₂ suggests that most current terrestrial vegetation models could be overestimating the magnitude of plausible C sequestration in a future CO₂-rich world. The trends of the dendroecological data reviewed here suggest the importance of multiple interactive effects in determining the net effect on plant growth and that caution is necessary when drawing conclusions from single-factorial experiments, particularly when estimating the future potential of terrestrial carbon sequestration.

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REFERENCES


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