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Twentieth century changes of tree-ring δ^{13}C at the southern range-edge of *Fagus sylvatica:* increasing water-use efficiency does not avoid the growth decline induced by warming at low altitudes

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Abstract

We aimed to gain knowledge on the changes in intrinsic water use efficiency (iWUE) in response to increasing atmospheric CO_2 concentrations and climate change over the last century. We investigated the variation in the iWUE of mature *Fagus sylvatica* trees located in the higher, central and lower altitudinal forest limits (HFL, CFA and LFL) of one of the southernmost sites of beech distribution in Europe, the Montseny Mountains in Catalonia (northeast Spain), during the last century by analysing the δ^{13}C of their tree rings. Pre- and post-maturation phases of the trees presented different trends in δ^{13}C, Δ^{13}C, Ci (internal CO_2 concentration), iWUE and basal area increment (BAI). Moreover, these variables showed different trends and absolute values in the LFL than in the other altitudinal sites, CFA and HFL. Our results show the existence of an age effect on δ^{13}C in the CFA and HFL (values increased by ca. 1.25‰ coinciding with the BAI suppression and release phases, previous to maturation). These age-related changes were not found in the LFL, whose beech trees arrived to maturation earlier and experienced drier conditions during the suppression phase. In the last 26 years of comparable mature trees, the increase of iWUE deduced from the Δ^{13}C analyses was ca. 10% in LFL, ca. 6% in CFA and not significant in HFL. These results show that climate change towards more arid conditions accounted for these higher Δ^{13}C-values and increases in the LFL more than the continuous increase in atmospheric CO_2 concentrations. This increased iWUE in the LFL did not avoid a decline in growth in these lowest altitudes of this beech southern range-edge as a result of warming. Furthermore, since there was no apparent change in iWUE and growth in the beech forests growing in the more standard-adequate environments of higher altitudes in the last 26 years, the rate of sequestration of C into temperate ecosystems may not increase with increasing atmospheric CO_2 concentrations as predicted by most models based on short-term small scale experiments.

Keywords: δ^{13}C, basal area increment, climate change, CO_2, drought, European beech, *Fagus sylvatica*, geographical range, Mediterranean, range edge

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Introduction

Changes in gas exchange and growth are among the primary responses of trees to environmental variations such as current changes in atmospheric CO_2 concentrations and climate change (Hughes, 2000; Körner, 2000). Experimental results (Morison, 1993; Picon *et al.*, 1996) indicate that plants are able to increase their water-use efficiency (WUE) as CO_2 levels rise. However, short-term experimental results from studies of plants in growth chambers, may not be reliable to predict the behaviour of mature trees growing in their natural environment, as trees are able to adjust their physiolo-

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gical response over time to gradually increasing CO_2 concentrations. Variation in intrinsic WUE (iWUE) estimated as the ratio between photosynthesis and stomatal conductance is recorded in the variation of the carbon isotope discrimination Δ^{13}C 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 Δ^{13}C, which will give insight into how naturally growing trees respond, or have responded, to increasing atmospheric CO_2 concentrations.

Several studies of this nature have been carried out on freely growing trees in the United States (Marshall & Monserud, 1996; Feng, 1998, 1999; Tang *et al*., 1999), Canada (Watmough *et al*., 2001), Tasmania (Francey & Farquhar, 1982), France (Bert *et al*., 1997; Duquesnay *et al*., 1998) and northern Europe (Waterhouse *et al*., 2004) and have revealed that trees vary in their responses to increasing atmospheric CO_2 concentrations. The apparent reason is that the carbon isotope discrimination Δ^{13}C in tree rings also responds strongly to many other environmental variables, especially climatic ones, such as growing season temperature, relative humidity and precipitation [see Switsur & Waterhouse (1998) and references therein]. Climate has changed in the last decades towards warmer conditions (IPCC, 2007a). These warmer conditions have strongly affected plant performance (Peñuelas & Filella, 2001; IPCC, 2007b). The long-term changes in the gas exchange metabolism of established trees must be occurring also in response to this climate change.

In this study, we used tree-ring carbon isotope analysis and the resulting calculation of Δ^{13}C to investigate iWUE changes during the past century in beech trees growing in the Montseny Mountains (Catalonia, NE, Spain). Beech saplings have been found to increase their iWUE when growing at elevated CO_2 concentration (Overdieck & Forstreuter, 1994). Beech shows a stronger iWUE response than other species such as oaks or pines, which may be a result of the particularly drought-sensitive nature of this species, caused, at least in part, by a generally relatively shallow rooting depth (Waterhouse *et al*., 2004; Lebourgeois *et al*., 2005). Moreover, we already know that beech forests in the Montseny Mountains, which are located in one of the southernmost distribution range-edges of this species, are particularly sensitive to climate change. Temperature has risen by ca. 1.4 °C in the last 50 years with no increase in precipitation in these mountains (Peñuelas & Boada, 2003). Increased discoloration and defoliation (Peñuelas & Boada, 2003) and declining growth and recruitment related to warming (and resulting aridification) have been reported for the *Fagus sylvatica* trees at the lower altitudinal *Fagus* forest limit of these mountains (Jump *et al*., 2006, 2007; Peñuelas *et al*., 2007). In addition to assessing temporal changes, we conducted an altitudinal survey by comparing the warmer and drier lower *Fagus* forest limit (LFL) with the cooler and wetter central forest area (CFA) and upper high *Fagus* forest limit (HFL). Since CO_2 has increased equally in these three sites, by comparing them we aimed to gain knowledge on the interaction of elevated CO_2 with warming (climate change) in altering WUE throughout the last decades. We asked whether changes in climate or CO_2 concentration were more important to beech tree growth, and how this was altered by altitudinal position. We also aimed to characterize the carbon isotope discrimination changes associated to the ageing of the trees and how are they affected by climate. Finally, we aimed to relate the changes in Δ^{13}C and iWUE with the growth changes, and to test whether possible increases in iWUE produced by increasing atmospheric CO_2 concentrations and by warming (and drought) can compensate or not the negative drought effects on growth measured as basal area increment (BAI).

Materials and methods

Study sites and sampling trees

This work was conducted at the southern edge of the distribution of *F. sylvatica* in Europe, in the Montseny Mountains, 50 km north–northwest of Barcelona (Catalonia, NE, Spain) (Fig. 1). *F. sylvatica* forest occurs in the temperate zone of the mountains, typically above 1000 m a.s.l. Below this, the vegetation is Mediterranean, dominated by *Quercus ilex* L. (holm oak) forest. *F. sylvatica* forms the treeline on the highest peaks of the region (Turó de l'Home and Les Agudes 1707 and 1706 m a.s.l., respectively). The *F. sylvatica* forest is a naturally occurring uneven-aged high forest that has been managed at low intensity by the selective removal of large trees, coupled with natural regeneration from seed. However, the impact of forest management on the upper and lower limits of the *F. sylvatica* forest has been low (Peñuelas & Boada, 2003). Soils of the Montseny *F. sylvatica* forest are typically Dystric Regosols and Dystric Cambisols established over schist and granodiorite lithology. The most typical forest community is the *Luzulo-Fagetum* with less common areas of *Heleboro-Fagetum*. A detailed description of the vegetation of Montseny and its altitudinal zonation is presented by Bolòs (1983).

Climate data (mean annual temperature and total precipitation) were taken from the Turó de l'Home meteorological station (1707 m a.s.l.), directly above the highest sample site (1640 m a.s.l.). Mean annual

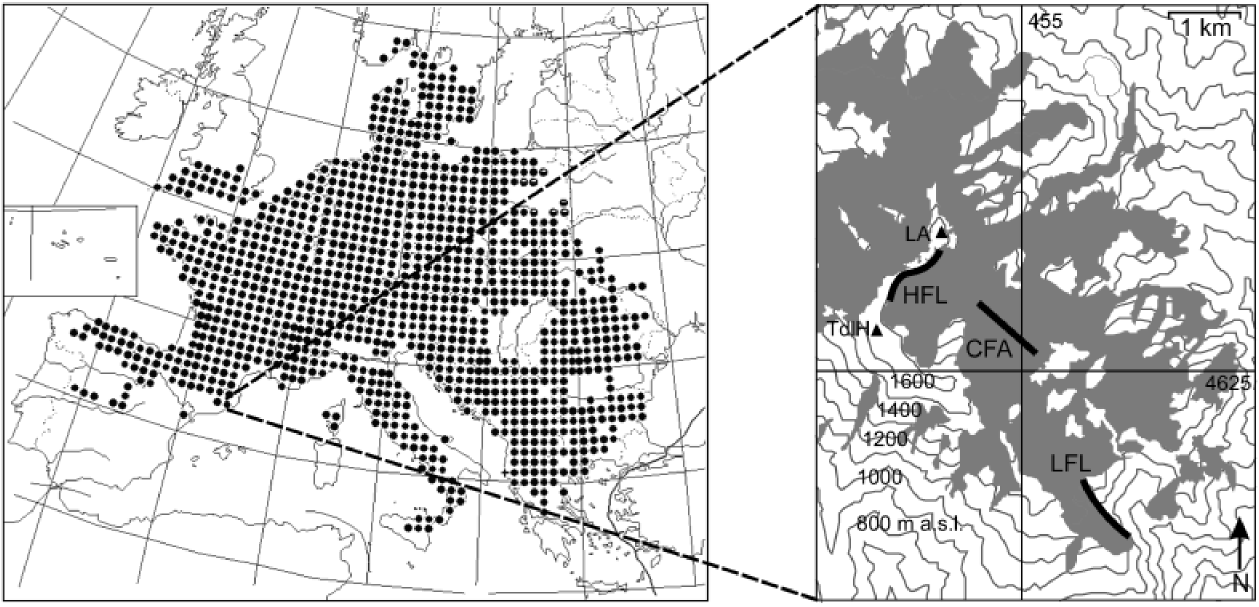

Fig. 1 Left: native distribution of *Fagus sylvatica* [reproduced from Jalas & Suominen (1976), by permission of the Committee for Mapping the Flora of Europe and Societas Biologica Fennica Vanamo]. Right: location of study sites in relation to *F. sylvatica* distribution in the study area. *F. sylvatica* distribution is shown in grey. Study sites: HFL upper treeline (high *Fagus* forest limit), CFA central forest area, LFL low *Fagus* forest limit. Bold lines show the distribution of individuals sampled at each site. Principal peaks are marked with a triangle: TdlH Turó de l'Home, LA Les Agudes. Grid lines are numbered with UTM coordinates in kilometres.

temperature and total annual precipitation records were available for the period 1952–2003 (Fig. 2).

Five mature dominant *F. sylvatica* trees (>80 years old), without signs of physical damage, were selected over a distance of 1 km at each of three altitudinal sites within a large area of continuous forest on the slopes on the southeast side of the Turó de l'Home ridge. These sites are identified here as the upper treeline (high *Fagus* forest limit or HFL; 1610–1665 m altitude), CFA (1105–1170 m altitude) and low *Fagus* forest limit (LFL; 940–1040 m altitude) (Fig. 1). The 648 m altitudinal range covered by these samples equates to a mean temperature difference of 3.3 °C between the HFL and LFL sites based on the altitudinal lapse rate of 0.51 °C per 100 m reported by Peñuelas & Boada (2003) for Montseny. Consequently, we adjusted temperature according to the mean altitude of each sample site. All three sampling sites were situated on the same southeast side of the Turó de l'Home-Les Agudes ridge. These sites were in closed forest in areas without signs of recent disturbance. The HFL and LFL sites were on steep rocky slopes, whereas the CFA site was predominantly on level ground with deeper, less stony soils around the Santa Fe river. The reason for doing this originally was to pick a site where there was no immediately obvious climatic limitation – (i.e. water availability could have been low if we had chosen a steep rocky section of central altitude). Although the soils are deeper in the CFA, the plant community is still the same and the plant community is a good indicator that the environmental conditions (particularly soils) are fairly similar. We, thus, studied a gradient covering the full species altitudinal distribution in this region in an area with available local climate data, with sites located on a single mountainside with no more than 5 km distance between samples, with same soils and plant community, with well-known forest history, and with supporting forest inventory data.

Sample preparation and analysis

Two or three increment cores were taken from each tree at breast height (1.3 m) using a 4.3 mm increment borer. Cores were placed into groove boards, wrapped with string to prevent twisting and left to air dry. When dry, the cores were sanded to prepare them for tree-ring analysis using standard dendroecological methods. Prepared samples were scanned at 1600 d.p.i. using a flatbed scanner and saved as .jpg files. Ring width was measured to an accuracy of 0.001 mm using COORECORDER v2.3.13 (Larsson, 2003). Preliminary matching of cores taken from the same tree was performed in CDENDRO v4.1.1. (Larsson, 2003) and cross-dating accuracy checked using COFECHA v6.06P (Grissino-Mayer,

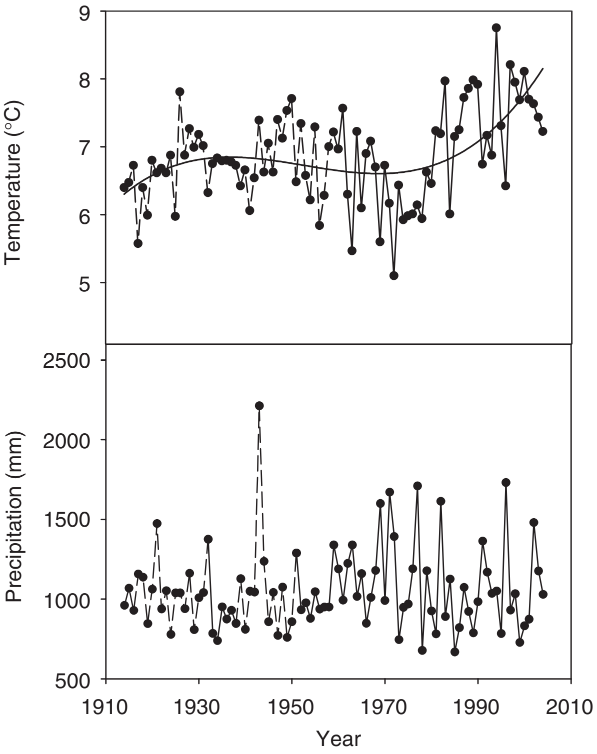

Fig. 2 Mean yearly temperature and total yearly precipitation 1914–2003 for Turó de l'Home (solid lines, data from climate records; dashed lines, data interpolated from neighbouring climate stations. See Jump *et al*., 2008). The recent rapid increase in mean annual temperature is highlighted by a cubic spline curve ($y = -110\,898.4650 + 170.4091x - 0.0873x^2 + 1.4898 \times 10^{-5}x^3$, $r^2 = 0.28$, $P < 0.001$). No long-term trend was observed in precipitation data, ($P = 0.60$).

2001; Holmes, 2001). Tree-ring data were standardized and data combined to produce a single chronology for each site using ARSTAN v6.05P (Holmes, 2001); for further details see Jump *et al*. (2008).

The longest cores were chosen which had a good (0.66 ± 0.028 SE) correlation with the master series for each site based on the standard chronologies reported by ARSTAN. Each core was then divided into 3-year blocks. The boundary layer of the third successive annual ring was marked on each core using a needle. This was carried out under a microscope and using images recorded from COORECORDER to ensure that no false rings were included in the analysis. The blocks were cut using a scalpel blade, coarsely ground in a mill (Moulinex coffee grinder, 60 g capacity, 180 W motor, Barcelona, Spain) and transferred to eppendorf tubes. The wood particles were then ground in a mixer mill (Tissue Lyser, Qiagen Inc., Valencia, CA, USA), using two tungsten beads, to achieve a finely ground homogenized sample. Eppendorfs were immersed in liquid nitrogen and then processed in the Tissue Lyser for two consecutive periods of 90 s (at 30 revolutions per second) with the samples being re-frozen in between the two grinding periods.

A subsample was taken from each of the eppendorf tubes and weighed on a Mettler Toledo MX5 Microbalance (average weight 0.841 mg, weight range 0.758–0.960 mg). Each subsample was transferred to a tin capsule (Lüdi AG, Flawil, Switzerland 0.03 mL; 3.3 mm × 1.5 mm), sealed by compressing the cup between forceps and then stored in a 96-well plate ready for analysis. One duplicate sample was prepared at every 12th sample and a 'blank' (i.e. empty tin cup) every 15th–18th sample. Standards were prepared in the same way as the wood samples by weighing between 0.6 and 0.8 mg of atropine or IAEA CH3, 6 and 7 in tin capsules. An elemental analyser Flash 1112 (Thermo Finnigan, MAT, Bremen, Germany) coupled to a Delta C isotope ratio mass spectrometer via a CONFLO III interphase (Thermo Finnigan MAT) was used for the ^{13}C isotope analyses. Accuracy was 0.2‰. Results were expressed as delta δ^{13}C‰ corrected by linear regression with international standard.

In the first 10–50 years of growth, when the tree is close to the forest floor, there is uptake of respired CO_2 and, therefore, C fixation is altered in these years. Wood formed during the first years of tree life is generally depleted in ^{13}C in comparison with wood formed later (Francey & Farquhar, 1982; Bert *et al*., 1997) in what is called the age effect. The corresponding δ^{13}C increase has been reported to increase around 1.5%. We only used the last 26 years of data to deduce the changes due to altered prevailing conditions.

Δ^{13}C and calculation of water use efficiency

We thereafter calculated Δ^{13}C, Ci and iWUE. The Δ^{13}C (carbon isotopic discrimination) was calculated as

$$\Delta^{13}\mathrm{C} = (\delta\mathrm{air} - \delta\mathrm{plant})/(1000 - \delta\mathrm{plant}) \times 1000,$$

following Farquhar *et al*. (1982) and using published values for air δ^{13}C from ice core measurements, direct atmospheric measurements and inferred from C4 plants (McCarroll & Loader, 2004). Since Δ^{13}C is related to C_i (intercellular CO_2 concentration) and C_a (ambient CO_2 concentration) by the following equation:

$$\Delta^{13}\mathrm{C}‰ = a + (b - a)(C_i/C_a),$$

where a is the discrimination against $^{13}CO_2$ during CO_2 diffusion through stomata ($a = 4.4$‰, O'Leary, 1981), b is the discrimination associated with carboxylation ($b = 27$‰, Farquhar & Richards, 1984) and given Fick's Law:

$$A = g_{CO_2}(C_a - C_i),$$

where A is the net photosynthesis, measured as CO_2 uptake, and g_{CO_2} is the leaf conductance to CO_2, and given that g_{H_2O}, the leaf conductance to water vapour is 1.6 g_{CO_2}, $\Delta^{13}C$ can be finally related to the ratio A/g_{H_2O} (iWUE) (Osmond *et al.*, 1980) by the following equation:

$$\Delta^{13}C = a + (b - a)(1 - 1.6A/C_a g_{H_2O}).$$

BAI measurements

Fifteen mature, dominant or co-dominant *F. sylvatica* trees without signs of physical damage were selected at each one of the three altitudinal sites and yearly tree BAI calculated as described in Jump *et al.* (2006). Ring width in mature trees declines with age; thus, if a declining growth trend is suspected, it may be impossible to investigate it on the basis of changes in ring width alone (Phipps & Whiton, 1988). The conversion of radial increment (ring width) into BAI overcomes this problem (Phipps & Whiton, 1988; LeBlanc, 1990b; Pedersen, 1998). Unlike ring width, age-related trends in unstandardized BAI are generally positive, culminating in a linear phase of high mature BAI that can be maintained for many decades (Phipps & Whiton, 1988; LeBlanc, 1990b). BAI series for dominant and co-dominant trees in mature stands typically show a period of early growth suppression (suppression phase) before a rapid increase in annual basal area growth (release phase). BAI may continue to increase in mature healthy mature trees (Phipps & Whiton, 1988; LeBlanc, 1990a, b; Duchesne *et al.*, 2002, 2003; Fekedulegn *et al.*, 2003; Muzika *et al.*, 2004), or stabilize (LeBlanc *et al.*, 1992), but it does not show a decreasing trend until trees begin to senesce (LeBlanc, 1990a; Duchesne *et al.*, 2002, 2003). Therefore, a negative trend in BAI is a strong indication of a true decline in tree growth (LeBlanc, 1990b; Pedersen, 1998; Jump *et al.*, 2006). During the suppression and release phases, growth is strongly affected by variation in light levels and is, thus not easily compared between trees. Consequently, we focused only on variation between levels of growth during the mature phase of growth, marked by the abrupt change in slope (levelling off) after the release phase.

Data treatment and statistical analysis

Regression analyses were used to identify significant temporal trends in $\delta^{13}C$, $\Delta^{13}C$, Ci, iWUE and BAI of individual trees over the last century and over the 26 years of mature trees common to all three chronologies (for the average chronology at each site). ANCOVA was used to test for differences between mean mature trees $\delta^{13}C$, $\Delta^{13}C$, Ci, iWUE and BAI at each site using the year as covariable. For correlation analyses of these variables with climate, current year mean annual temperature, total annual precipitation, Gaussen index of aridity (temperature/2Precipitation) and atmospheric CO_2 concentrations were calculated for the period beginning October of the previous year and ending with September of the current year. This procedure avoids relating growth to climate in the months after leaf fall when growth has ceased. Regression, ANCOVA, correlation and all the statistical calculations and analyses were conducted using the STATISTICA software package (StatSoft Inc., Tulsa, OK).

Results

Temporal and altitudinal trends of $\delta^{13}C$, $\Delta^{13}C$, Ci, iWUE and BAI

Two different trends in $\delta^{13}C$, $\Delta^{13}C$, Ci and BAI were found throughout the tree chronologies corresponding to the pre and postmaturation phases. Moreover, these trends as well the absolute values were different in the LFL than in the other altitudinal sites, CFA and HFL.

During the first stage of their life before reaching maturity in the 1970s, the values of $\delta^{13}C$ of tree-rings increased from −27.3‰ to −26‰ in both HFL and CFA, but remained without significant changes between −26‰ and −25‰ in the LFL. Isotopic discrimination ($\Delta^{13}C$) significantly decreased from ca. 20‰ to ca. 18.5‰ in the HFL and CFA, whereas it remained at ca. 18‰ throughout the time in LFL (Fig. 3). Ci was also lower, between 190 and 200 ppm, in the LFL than in the CFA and HFL (215 and 210 ppm, respectively). The iWUE increased from ca. 55 to ca. 75 $\mu mol\, mol^{-1}$ in the HFL and the CFA, whereas it was already 70–75 $\mu mol\, mol^{-1}$ in the LFL since the beginning of the measuring period (Fig. 3). Following low BAI during the suppression phase, the BAI curve showed a rapid increase during release before levelling off to mature tree levels of growth. This levelling off marks the beginning of the mature phase (Fig. 3). Figure 3 shows that the suppression phase of BAI was less severe at the HFL than in the CFA. Although there was no significant difference in the tree mean age or diameter at each site (Table 1), the release phase began much earlier at the LFL site than at higher altitudes as also evidenced in the $\delta^{13}C$, $\Delta^{13}C$, Ci and iWUE temporal trends (Fig. 3).

After maturation of all studied trees, when there was no age effect, (i.e. from 1978 to 2003), during the 26 years of mature trees common to all three chronologies, $\delta^{13}C$ started to decrease again up to −26.7‰ (LFL), −27.1‰ (CFA) and −27.5‰ (HFL). $\Delta^{13}C$ increased (from 18.2‰ up to ca. 18.9‰) only in the CFA. Ci increased in all three altitudinal sites, from 208 to 235 $\mu mol\, mol^{-1}$ in LFL, from 215 to 240 $\mu mol\, mol^{-1}$ in CFA and from 211 to

245 µmol mol^{-1} in HFL (Fig. 3). The iWUE increased by 10% in the LFL and by 6% in the CFA, did not significantly change in the HFL. BAI decreased by 33% in the LFL (Fig. 3). There was no evidence for any increasing or decreasing BAI trend of trees at higher altitudes, in the CFA and HFL sites. In this period with mature

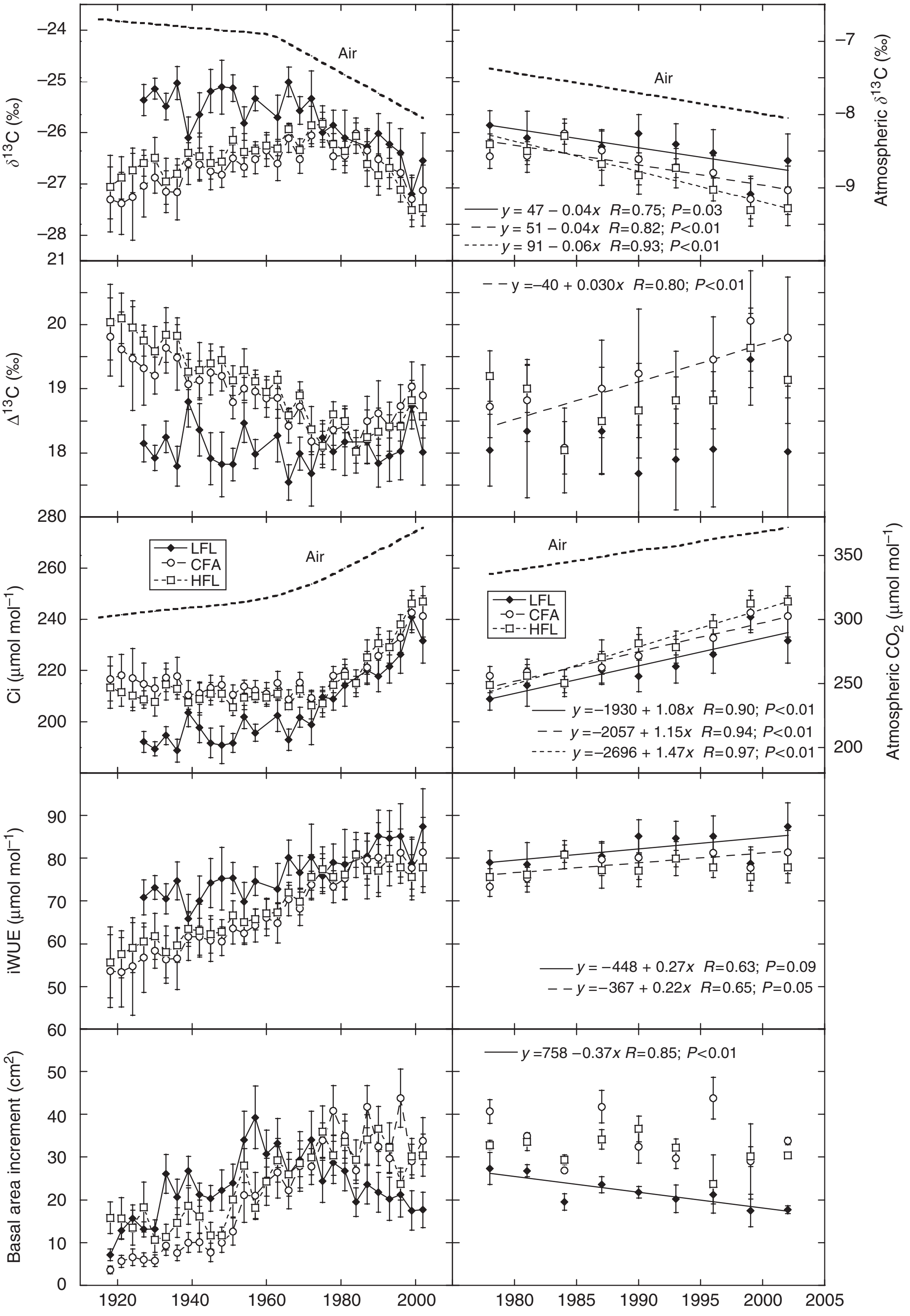

Table 1 Summary information for *Fagus sylvatica* samples used for the Δ^{13}C and BAI measurements

Site	Stand basal area ($m^2\,ha^{-1}$)	Leaf area index	Altitude (m a.s.l.)	*N*	DBH (cm)	Age (years)
Upper treeline (HFL)	23.98	4.06	1640	5 (15)	56.6 (28.2–118.4)	96.2 (50–229)
Central forest area (CFA)	25.24	4.30	1127	5 (14)	47.3 (25.8–66.2)	94.6 (57–143)
Low *Fagus* limit (LFL)	20.62	4.09	992	5 (14)	51.3 (33.3–68.1)	90.6 (50–119)

Altitude, mean sample altitude; *N*, number of trees analysed for Δ^{13}C, with the number of trees analysed BAI between brackets; DBH, diameter at breast height of all analysed trees; age, age at breast height. Mean values for DBH and age are followed by the range in italics. DBH and age do not differ significantly between sites (Kruskal–Wallace test, $P > 0.05$).

trees, (i.e. from 1978 to 2003), the Δ^{13}C, the Ci and the BAI were lower and the iWUE higher in the LFL than in the CFA and the HFL (ANCOVAs, $P < 0.01$) (Fig. 4).

Δ^{13}C and BAI relationships with atmospheric CO_2 and climate

Figure 5 shows the different age effect on δ^{13}C, Δ^{13}C, Ci, iWUE and BAI in the LFL than in the other two sites, CFA and HFL, by relating the changes of all these carbon isotope-derived variables and BAI with the atmospheric CO_2 concentrations. This figure also shows how LFL beech trees grew less in the mature phase and increasingly reduced BAI despite presenting higher and continuously increasing iWUE.

The estimated iWUE of mature trees was positively correlated with atmospheric CO_2 concentrations in LFL and CFA, although with lower slope in the latter, but was not in the HFL, and it was also positively correlated with the temperature in the LFL (Fig. 6). BAI was negatively correlated with both the atmospheric CO_2 concentration and the temperature also only in the LFL (Fig. 7). Thus, BAI decreased in the LFL in spite of increasing atmospheric CO_2 concentration, Ci and iWUE (Figs 5 and 7). The relationships of all these variables with the precipitation and Gaussen aridity index were always weaker than those with temperature or CO_2 (data not shown).

Discussion

The first decades of growth: age effect

Our results show the existence of an age effect on carbon isotope discrimination in the CFA and HFL (δ^{13}C values increased by ca. 1.25‰ coinciding with the suppression and release phases in BAI). This age effect may be attributed to two different causes: changes in micro-environmental variables during stand maturation and physiological changes linked to tree structural development. δ^{13}C increases with an increase in vapour pressure deficit (Farquhar *et al.*, 1989) or irradiance (Zimmermann & Ehleringer, 1990), both variables that change as trees grow; trees may also assimilate more soil-respired and therefore isotopically lighter CO_2 during stand maturation (Schleser & Jayasekera, 1985). Among the physiological changes linked to tree structural development there are changes in hydraulic conductivity (McCarroll & Loader, 2004) or/and bark re-fixation of respired CO_2 (Cernusak *et al.*, 2001) that may add to this increase in δ^{13}C. The age effect denoted by δ^{13}C values was in agreement with the age changes in BAI. Trees at the HFL and CFA showed a similar pattern for δ^{13}C and BAI. Following low BAI during the suppression phase, the BAI curve showed a rapid increase during release before levelling off to mature tree levels of growth. This levelling off, that marked the beginning of the mature phase, was a likely consequence of competition between mature canopy trees (LeBlanc, 1990a, b). These age-related changes were not found in the LFL, whose beech trees reached maturity earlier. Their less negative δ^{13}C values seem the result of warmer and drier conditions in the LFL in the first decades of growth. Since more negative leaf δ^{13}C values are recorded in more closed canopies than in more open stands (Leavitt, 1993; Buchmann *et al.*, 1997) and strong interacting effects of water availability and light as depending on stand density on δ^{13}C have been shown for beech (Gessler *et al.*, 2001), the less negative LFL δ^{13}C values in the first decades of growth might also indicate different stand story and structure. However, these were very similar in the three altitudinal sites, at least

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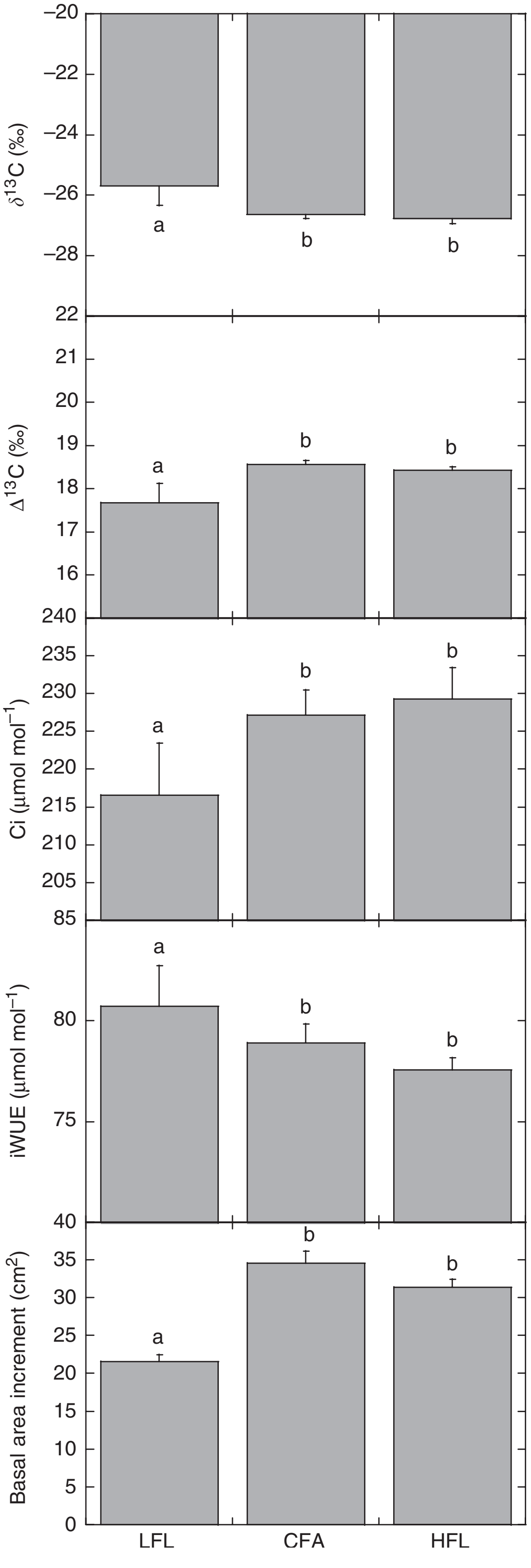
Fig. 3 Left columns, full chronology of mean *Fagus sylvatica* δ^{13}C, Δ^{13}C, Ci, intrinsic water use efficiency (iWUE) and basal area increment (BAI) for the three forest sites differing in altitude. HFL upper tree-line (high *Fagus* forest limit), CFA central forest area, LFL low *Fagus* forest limit. Right columns, temporal trends of the same variables in mature trees (26 years from 1978 to 2003) at each altitudinal site. The significant trends are represented by lines with the corresponding equation, coefficient of correlation and statistical significance. In all cases, 3-year pooled data is depicted.

in the final period of tree maturity compared in this study (last 26 years of mature trees) (Table 1). Changes in δ^{13}C over time and among sites could also occur as a result of changes in the composition of the wood (i.e. changes in the cellulose to lignin ratio) as different chemical compounds are known to have different carbon isotope signatures. The changes in CO_2 concentration or meteorological variables might affect the chemical composition of the wood. We did not analyse such possible changes in chemical composition but, in any case, any potential change in composition of the wood does not explain why the time series differ since there is no apparent reason why there should be any difference in CO_2 or meteorological trends only applying to the LFL and not the CFA and HFL also.



Diminishing increases in iWUE: CO_2, drought and other factors

Over the whole period of study, the iWUE increased ca. 50% in the CFA and HFL sites which is in agreement with the 44% increase reported by Duquesnay *et al.* (1998) in their study of the changes in tree-ring δ^{13}C and WUE of beech trees in northeastern France. Although in both cases an important part of the change is linked to the age effect in the first decades of life, these increases in iWUE are in agreement with previous reports (Peñuelas & Azcón-Bieto, 1992; Woodward, 1993; Ehleringer & Cerling, 1995; Feng & Epstein, 1995; Bert *et al.*, 1997; Duquesnay *et al.*, 1998; Feng, 1999) that found a decrease in carbon isotopic discrimination Δ or an increase of WUE during the same period of the 20th century by using historical records both in leaves and tree-rings. Isotopic discrimination Δ^{13}C of herbarium leaves of Mediterranean trees decreased by 1‰ and thus iWUE increased during the last decades of 20th century (Peñuelas & Azcón-Bieto, 1992). Woodward (1993) found increases in iWUE by 30% since 1930 also using herbarium leaves. Others have also found this increased iWUE in tree-rings (Feng & Epstein, 1995; Bert *et al.*, 1997; Feng, 1999) although some (Marshall & Monserud, 1996) did not find such changes. In addition, in herbarium specimens, an average reduction in stomatal density of many tree species has been found over the past century (Woodward, 1987; Peñuelas & Matamala, 1990), although for beech trees, different responses have been reported, from 6% increase to a 43% decrease during the last 200 years (Paoletti & Gellini, 1993; Woodward & Kelly, 1995).

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Fig. 4 Mean *Fagus sylvatica* δ^{13}C, Δ^{13}C, Ci, intrinsic water use efficiency (iWUE) and basal area increment (BAI) of mature beech trees in the three forest altitudinal sites during the period 1978–2003. Different letters indicate significantly different values between sites ($P < 0.05$; *post hoc* Fisher test, after ANCOVA analyses).

The graph of IWUE plotted against atmospheric CO_2 concentrations in mature trees (Fig. 6) shows that the rate of increase in iWUE diminished from LFL to CFA until vanishing (there was no increase) at HFL, thus indicating decreasing sensitivities to increasing levels of CO_2 in the sites not yet water-stressed by increased aridification. Such behaviour is not observed in growth chamber experiments nor predicted by current models, which predict a continuing rise in terrestrial carbon storage as a result of the combined effects of CO_2 fertilization and climate change as CO_2 concentration increases (Melillo *et al.*, 1996; Cao & Woodward, 1998; Betson *et al.*, 2007; Leuzinger & Körner, 2007). If a long-term or permanent reduction in sensitivity to atmospheric CO_2 such as this one is established for a significant proportion of trees in temperate regions, the degree of future terrestrial carbon storage would have been overestimated.

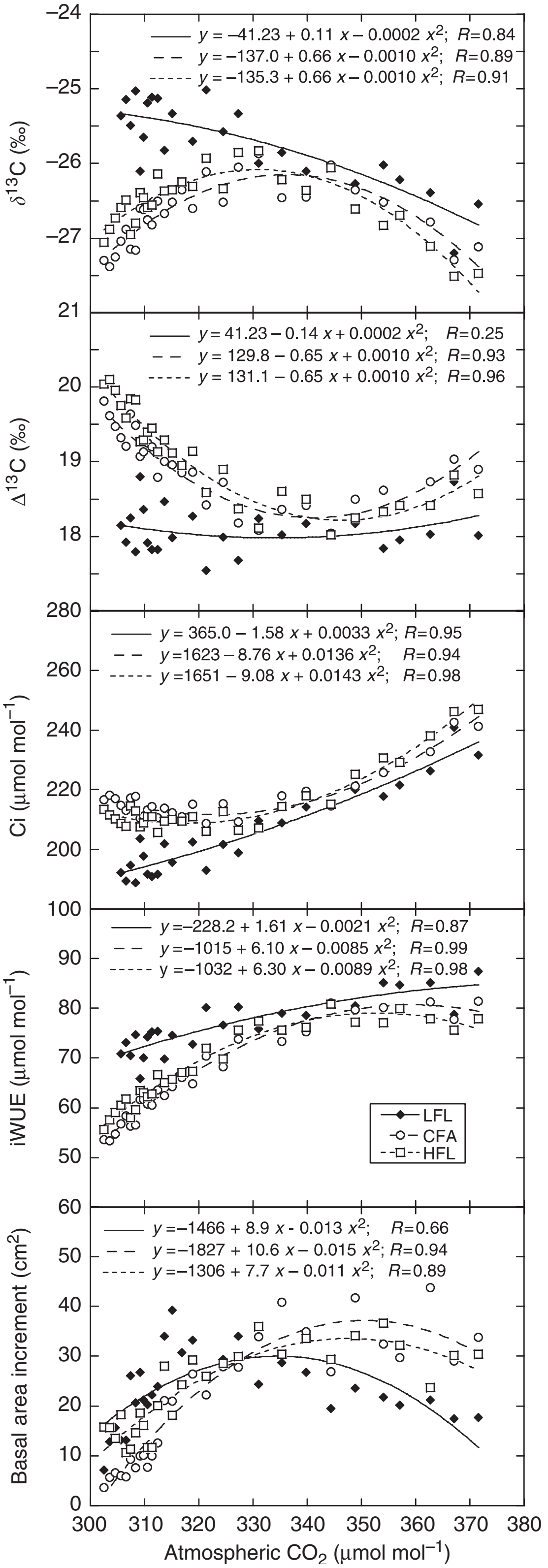

Fig. 5 Mean *Fagus sylvatica* $\delta^{13}C$, $\Delta^{13}C$, Ci, intrinsic water use efficiency (iWUE) and basal area increment (BAI) relationships with atmospheric CO_2 concentrations changing during the 20th century for the three forest sites differing in altitude. HFL, upper tree-line (high *Fagus* forest limit); CFA, central forest area; LFL, low *Fagus* forest limit. In all cases, 3-year pooled data is depicted.

For the last 26 years, the iWUE of the comparable mature trees in the sites with adequate temperature and water availability either did not increase (HFL) or only increased by 6% (CFA). However, in the warm and dry LFL, iWUE increased by 10%. Since the atmospheric CO_2 has increased similarly in the three sites, the higher increase in iWUE in this LFL site seems attributable to the warming and corresponding stronger drought stress generated by an increasing warming not accompanied by increased precipitation and, therefore, a climate change towards more arid conditions (Peñuelas *et al.*, 2002). During the period of study, there were also significant changes in many other additional variables including for example nitrogen deposition (Rodà *et al.*, 2002), atmospheric pollution (Ribas & Peñuelas, 2004), or possibly cloudiness and fogginess that might have also accounted for another part of these changes in iWUE.

Declining growth at lower altitudes

Apart from different trends over time, the LFL always presented higher $\delta^{13}C$, lower discrimination $\Delta^{13}C$, and higher iWUE than the CFA and HFL (Fig. 4). The higher iWUE in LFL than in the other sites and its increase in the last 26 years together with the very sharp increase in internal CO_2 concentration resulting from the rise of atmospheric CO_2 concentration that should result in an increase in photosynthetic rates, were not sufficient to revert the decline in growth in the LFL. In contrast with the trees at the other CFA and HFL sites, and in spite

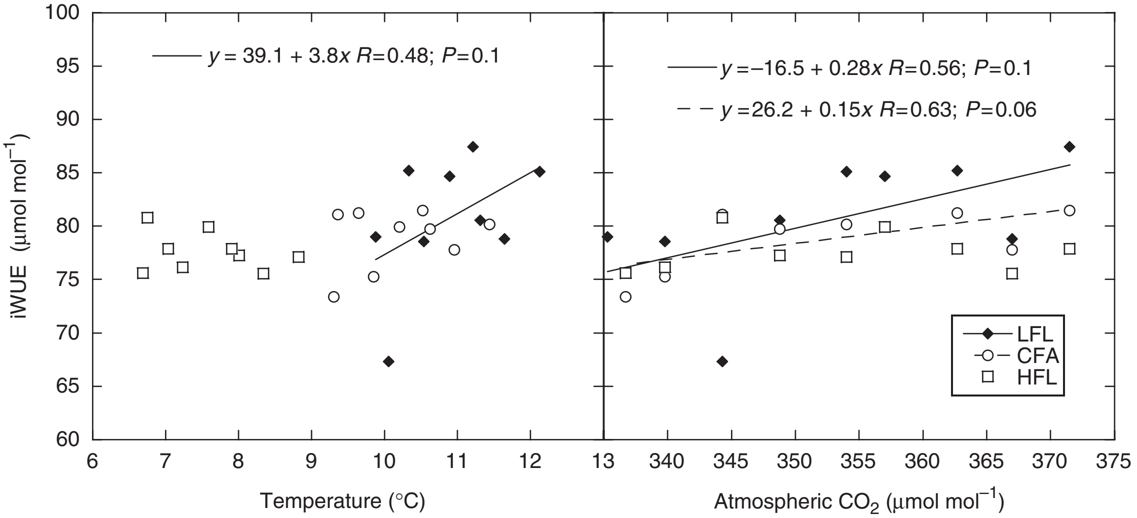

Fig. 6 Intrinsic water use efficiency (iWUE) relationships with temperature and atmospheric CO$_2$ concentrations in mature beech trees during the last period of study, 1978–2003. In all cases, 3-year pooled data is depicted.

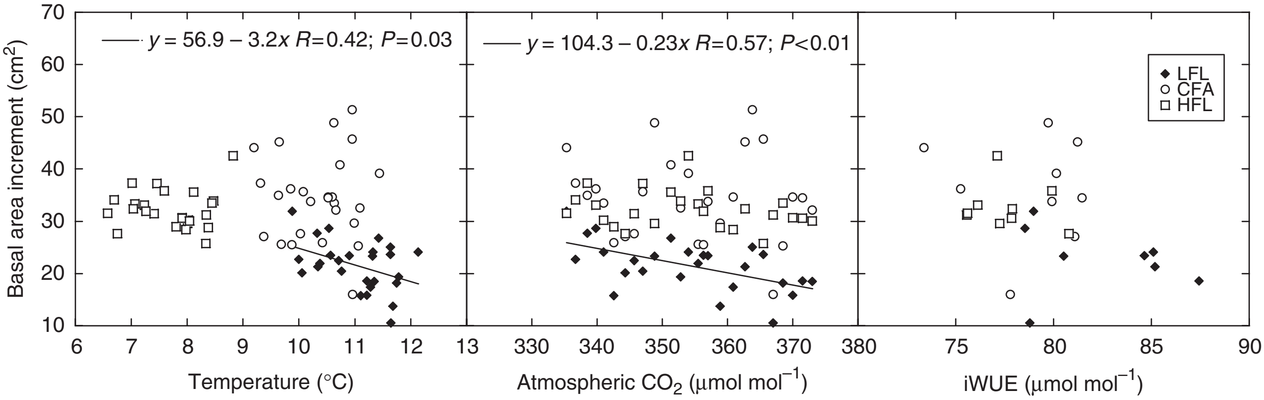

Fig. 7 Basal area increment (BAI) relationships with temperature, atmospheric CO$_2$ concentrations and intrinsic water use efficiency (iWUE) in mature beech trees during the last period of study, 1978–2003. Three-year poled data is depicted for iWUE.

that recent climatic warming has increased the length of the growing season for many species in these Montseny Mountains (Peñuelas *et al*., 2002), the LFL trees showed a consistent decline of mature tree BAI over the last 26 years. The declining BAI (negative slope) was not an expected consequence of tree maturation (LeBlanc *et al*., 1992). A negative BAI trend is a strong indicator of a decline in tree growth (Phipps & Whiton, 1988; LeBlanc, 1990a, b; Duchesne *et al*., 2002, 2003; Jump *et al*., 2006) that was not avoided by the increased WUE. The close correspondence of the declining BAI trend at the LFL with that of temperature (Fig. 7) shows a link between declining growth at the LFL and the rising temperatures of the Montseny region, in agreement with the climate-response reported for *F. sylvatica* in these Montseny Mountains in a previous dendroecological study by Gutierrez (1988) who found that growth of *F. sylvatica* at low altitudes was limited by high growing-season temperatures and promoted by high precipitation during the growing season. This is consistent with the high sensitivity of this species to drought, especially across the southern region of its distribution (Gutierrez, 1988; Dittmar *et al*., 2003; Lebourgeois *et al*., 2005) and in part explains the climate-growth responses seen in Figs 6 and 7, with the negative relationships between BAI and temperature. No such relationships were found in CFA and HFL which do not yet seem limited by temperature or precipitation or both. The CFA site is located in a level area of forest around the Santa Fe river and has deeper soils than those of the steep northeast-facing slope of the LFL [see Bolòs (1983)]. Lebourgeois *et al*. (2005) show that soil water deficits play a crucial role in limiting the growth of *F. sylvatica* and moderating the effects of high temperatures. Differences in soil water availability between the two sites may explain the differences in iWUE and why growth of the CFA remains unchanged whilst it is declining rapidly at the LFL. Increasing temperatures could produce higher respiration rates, which would lower NPP and, thus BAI. It is also possible that a decrease in g could cause an increase in WUE with no associated change (or even a decline) in A. A change in g could be caused by the increasing temperatures and hence water stress, or it can also occur that the direct effect of temperature could have a negative impact on the assimilation capacity of the leaves. In any case, the data show that an increase in iWUE does not necessarily increase the BAI.

The comparable decrease in BAI reported in other south-European regions (Piovesan *et al.*, 2005) suggests that this climate-related decline in the growth of *F. sylvatica* at the edge of its range in Montseny is not an isolated phenomenon, but may be occurring at the southern range-edge of this species elsewhere in Europe. *F. sylvatica* is an increasingly important species for European forestry (Dittmar *et al.*, 2003). Recent climatic warming has increased the length of the growing season for many species in the Montseny region (Peñuelas *et al.*, 2002), suggesting a positive growth response to recent climatic changes might be expected for *F. sylvatica*. Many studies in recent years describe increasing growth trends for *F. sylvatica* and other forest species throughout Europe (Sabaté *et al.*, 2002; Dittmar *et al.*, 2003; Bascietto *et al.*, 2004 and references therein), which may lead to the assumption that carbon sequestration by this species is increasing. Our results demonstrate that for some low-altitude and low-latitude populations the reverse may be true, with increasing temperatures resulting in recent and rapid decline of the growth of this species in spite of the increasing WUE.

The limitation of growth by drought at the low-altitude, low-latitude range-edge may have profound consequences for the persistence of populations where current increases in temperature are not matched by increases in precipitation such as it is occurring in the Montseny Mountains studied here. There are now numerous reports of ongoing climate-related range changes of woody species [see references in Jump *et al.* (2006)] including in the studied beech forests of Montseny Mountains (Peñuelas & Boada, 2003; Peñuelas *et al.*, 2008). However, these are heavily biased toward the leading edge of the species' distribution because recruitment is generally more sensitive to climate than mortality (LaMarche, 1973; Lloyd, 1997). Consequently, it may take much longer to detect range changes at the retreating edge than at the expanding edge, if the same survey-based methods are used to analyse both. These iWUE changes deduced from the tree-rings $\Delta^{13}C$ provide an early signal for changes in response to environmental disturbances such as the elevated CO_2 concentrations or climate change.

Conclusions

The changes in carbon isotope discrimination of the beech tree-rings showed increasing beech iWUE in the last decades in the lower altitudes implying that the ratio between assimilation rates and stomatal conductance has increased. No such changes were found in the higher altitude beech forests. The results presented here emphasize that the global rise in CO_2 but especially the changing climate are already influencing the gas exchange of beech forests at their most sensitive range edges. Among their effects, these resulting increases in WUE are not sufficient to reverse the declining growth linked to increasing temperatures and drought in the southernmost low altitude edge of their distribution. In addition, the results also show an insensitivity of WUE and growth to increasing atmospheric CO_2 concentrations in the central and higher altitude sites, (i.e. in the more adequate habitats for beech), which is in agreement with the suggestion of Waterhouse *et al.* (2004) and Betson *et al.* (2007), that such a phenomenon may represent a saturation effect, which, if proven, would mean that most current models of future plant CO_2 uptake overestimate the degree of C sequestration possible within the terrestrial biosphere.

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References

Bascietto M, Cherubini P, Scarascia-Mugnozza G (2004) Tree rings from a European beech forest chronosequence are useful for detecting growth trends and carbon sequestration. *Canadian Journal of Forest Research*, **34**, 481–492.

Bert GD, Leavitt SW, Dupouey JL (1997) Variations of wood $\delta^{13}C$ and water-use efficiency of *Abies alba* (Mill.) during the last century. *Ecology*, **78**, 1588–1596.

Betson NR, Johannisson C, Löfvenius MO *et al.* (2007) Variation in the $\delta^{13}C$ of foliage of *Pinus sylvestris* L. in relation to climate and additions of nitrogen: analysis of a 32 year chronology. *Global Change Biology*, **13**, 2317–2328.

Bolòs O (1983) *La vegetació del Montseny*. Diputació de Barcelona, Barcelona.

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.

Cao M, Woodward FI (1998) Dynamic responses of terrestrial ecosystem carbon cycling to global climate change. *Nature*, **393**, 249–252.

Cernusak LA, Marshall JD, Comstock JP *et al.* (2001) Carbon isotope discrimination in photosynthetic bark. *Oecologia*, **128**, 24–35.

Dittmar C, Zech W, Elling W (2003) Growth variations of Common beech (*Fagus sylvatica* L.) under different climatic and environmental conditions in Europe – a dendroecological study. *Forest Ecology and Management*, **173**, 63–78.

Duchesne L, Ouimet R, Houle D (2002) Basal area growth of sugar maple in relation to acid deposition, stand health and soil nutrients. *Journal of Environmental Quality*, **31**, 1676–1683.

Duchesne L, Ouimet R, Morneau C (2003) Assessment of sugar maple health based on basal area growth pattern. *Canadian Journal of Forest Research*, **33**, 2074–2080.

Duquesnay A, Breda N, Stivenard M *et al.* (1998) Changes of tree-ring delta C-13 and water use efficiency of beech (*Fagus sylvatica* L.) in north-eastern France during the past century. *Plant Cell and Environment*, **21**, 565–572.

Ehleringer JR, Cerling TE (1995) Atmospheric CO_2 and the ratio of intercellular to ambient CO_2 concentrations in plants. *Tree Physiology*, **15**, 105–111.

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

Farquhar GD, Richards RA (1984) Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. *Australian Journal of Plant Physiology*, **11**, 539–552.

Fekedulegn D, Hicks RR, Colbert JJ (2003) Influence of topographic aspect, precipitation and drought on radial growth of four major tree species in an Appalachian watershed. *Forest Ecology and Management*, **177**, 409–425.

Feng X (1998) Long-term c_i/c_a response of trees in western North America to atmospheric CO_2 concentrations derived from carbon isotope chronologies. *Oecologia*, **117**, 19–25.

Feng X (1999) Trends in intrinsic water-use efficiency of natural trees for the past 100–200 years: a response to atmospheric CO_2 concentrations. *Geochimica et Cosmochimica Acta*, **63**, 1891–1903.

Feng X, Epstein S (1995) Carbon isotopes of trees from arid environments and implications for reconstructing atmospheric CO_2 concentration. *Geochimica et Cosmochimica Acta*, **59**, 2599–2608.

Francey RJ, Farquhar GD (1982) An explanation of $^{13}C/^{12}C$ variations in tree rings. *Nature*, **297**, 28–31.

Gessler A, Schrempp S, Matzarakis A, Mayer H, Rennenberg H, Adams MA (2001) Radiation modifies the effect of water availability on the carbon isotope composition of beech (*Fagus sylvatica*). *New Phytologist*, **150**, 653–664.

Grissino-Mayer HD (2001) Evaluating crossdating accuracy: a manual and tutorial for the computer program COFECHA. *Tree-Ring Research*, **57**, 205–221.

Gutierrez E (1988) Dendroecological Study of *Fagus sylvatica* L. in the Montseny Mountains (Spain). *Acta Oecologica-Oecologia Plantarum*, **9**, 301–309.

Holmes RL (2001) Dendrochronology Program Library. Available from the Laboratory of Tree Ring Research, University of Arizona, Tucson, USA. http://www.ltrr.arizona.edu/software.html

Hughes L (2000) Biological consequences of global warming: is the signal already apparent? *Trends in Ecology and Evolution*, **15**, 56–61.

IPCC (2007a) *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (eds Solomon S, Qin D, Manning M *et al.*), Cambridge University Press, Cambridge, UK.

IPCC, Fischlin A, Midgley GF, Price JT *et al.* (2007b) Ecosystems, their propierties, goods, and services. In: *Climate change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (eds Parry ML, Canziani OF, Palutikof JP *et al.*), pp. 211–272. Cambridge University Press, Cambridge.

Jump AS, Hunt J, Peñuelas J (2006) Rapid Climate change-related growth decline at the southern range edge of *Fagus sylvatica*. *Global Change Biology*, **12**, 2163–2174.

Jump AS, Hunt J, Peñuelas J (2007) Climate relationships of growth and establishment across the altitudinal range of *Fagus sylvatica* in the Montseny Mountains, NE Spain. *Ecoscience*, **14**, 507–518.

Körner C (2000) Biosphere responses to CO_2 enrichment. *Ecological Applications*, **10**, 1590–1619.

LaMarche VC Jr (1973) Holocene climatic variations inferred from treeline fluctuations in the White Mountains, California. *Quaternary Research*, **3**, 632–660.

Larsson LA (2003) COORECORDER v2.3.13: Image Co-ordinate Recording Program. Available online at http://www.cybis.se

Leavitt SW (1993) Seasonal $^{13}C/^{12}C$ changes in tree rings: species and site coherence, and a possible drought influence. *Canadian Journal of Forest Research*, **23**, 210–218.

LeBlanc DC (1990a) Red spruce decline on Whiteface mountain, New York. I. Relationships with elevation, tree age and competition. *Canadian Journal of Forest Research*, **20**, 1408–1414.

LeBlanc DC (1990b) Relationships between breast-height and whole-stem growth indices for red spruce on Whiteface mountain, New York. *Canadian Journal of Forest Research*, **20**, 1399–1407.

LeBlanc DC, Nicholas NS, Zedaker SM (1992) Prevalence of individual-tree growth decline in red spruce populations of the southern Appalachian mountains. *Canadian Journal of Forest Research*, **22**, 905–914.

Lebourgeois FL, Bréda N, Ulrich E *et al.* (2005) Climate-tree-growth relationships of European beech (*Fagus sylvatica* L.) in the French Permanent Plot Network (RENECOFOR). *Trees*, **19**, 385–401.

Leuzinger S, Körner C (2007) Water savings in mature deciduous forest trees under elevated CO_2. *Global Change Biology*, **13**, 2498–2508.

Lloyd AH (1997) Response of tree-line populations of foxtail pine (*Pinus balfouriana*) to climate variation over the last 1000 years. *Canadian Journal of Forest Research*, **27**, 936–942.

Marshall JD, Monserud RA (1996) Homeostatic gas-exchange parameters inferred from $^{13}C/^{12}C$ in tree rings of conifers. *Oecologia*, **105**, 13–21.

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

Melillo JM, Prentice IC, Farquhar GD, Schulze ED *et al*. (1996) Terrestial biotic responses to environmental change and feedbacks to climate. In: *Climate Change 1995* (eds Houghton JT, Meira Filho LG, Callander BA *et al*.), pp. 445–481. Cambridge University Press, Cambridge.

Morison JIL (1993) Response of plants to CO_2 under water limited conditions. *Vegetatio*, **104**, 193–209.

Muzika RM, Guyette RP, Zielonka T *et al*. (2004) The influence of O_3, NO_2 and SO_2 on growth of *Picea abies* and *Fagus sylvatica* in the Carpathian mountains. *Environmental Pollution*, **130**, 65–71.

O'Leary M (1981) Carbon isotope fractionation in plants. *Phytochemistry*, **20**, 553–567.

Osmond CB, Bjorkman O, Anderson DJ (1980) *Physiological Processes in Plant Ecology. Toward a Synthesis With Atriplex*. Springer, New York.

Overdieck D, Forstreuter M (1994) Evapotranspiration of beech stands and transpiration of beech leaves subject to atmospheric CO_2 enrichment. *Tree Physiology*, **14**, 997–1003.

Paoletti E, Gellini R (1993) Stomatal density variation in beech and holm oak leaves collected over the last 200 years. *Acta Oecologica*, **14**, 173–178.

Pedersen BS (1998) The role of stress in the mortality of Midwestern oaks as indicated by growth prior to death. *Ecology*, **79**, 79–93.

Peñuelas J, Azcón-Bieto J (1992) Changes in leaf $\Delta^{13}C$ of herbarium plant species during the last 3 centuries of CO_2 increase. *Plant Cell and Environment*, **15**, 485–489.

Peñuelas J, Boada M (2003) A global change-induced biome shift in the Montseny Mountains (NE Spain). *Global Change Biology*, **9**, 131–140.

Peñuelas J, Filella I (2001) Phenology: response to a warming world. *Science*, **294**, 793–795.

Peñuelas J, Filella I, Comas P (2002) Changed plant and animal life cycles from 1952 to 2000 in the Mediterranean region. *Global Change Biology*, **8**, 531–544.

Peñuelas J, Matamala R (1990) Changes in N and S leaf content, stomatal density and specific leaf area of 14 plant species during the last three centuries of CO_2 increase. *Journal of Experimental Botany*, **41**, 1119–1124.

Peñuelas J, Ogaya R, Boada M *et al*. (2007) Migration, invasion and decline: changes in recruitment and forest structure in a warming-linked biome shift in Catalonia (NE Spain). *Ecography*, **30**, 830–838.

Phipps RL, Whiton JC (1988) Decline in long-term growth trends of white oak. *Canadian Journal of Forestry Research*, **18**, 24–32.

Picon C, Guehl JM, Aussenac G (1996) Growth dynamics, transpiration and water-use efficiency in *Quercus robur* plants submitted to elevated CO_2 and drought. *Annales des Sciences Forestières*, **53**, 431–446.

Piovesan G, Di Filippo A, Alessandrini A *et al*. (2005) Structure, dynamics and dendroecology of an old-growth *Fagus* forest in the Apennines. *Journal of Vegetation Science*, **16**, 13–28.

Ribas A, Peñuelas J (2004) Temporal patterns of surface ozone levels in different habitats of the North Western Mediterranean basin. *Atmospheric Environment*, **38**, 985–992.

Rodà F, Avila A, Rodrigo A (2002) Nitrogen deposition in Mediterranean forests. *Environmental Pollution*, **118**, 205–213.

Sabaté S, Gracia CA, Sánchez A (2002) Likely effects of climate change on growth of *Quercus ilex*, *Pinus halepensis*, *Pinus pinaster*, *Pinus sylvestris* and *Fagus sylvatica* forests in the Mediterranean region. *Forest Ecology and Management*, **162**, 23–37.

Schleser GF, Jayasekera R (1985) $\delta^{13}C$ variations in leaves of a forest as an indication of reassimilated CO_2 from the soil. *Oecologia*, **65**, 536–542.

Switsur R, Waterhouse J (1998) Stable isotopes in tree ring cellulose. In: *Stable Isotopes: Integration of Biological, Ecological and Geochemical Processes* (ed. Griffiths H), pp. 303–321. BIOS Scientific Publishers Ltd, Oxford.

Tang K, Feng X, Funkhouser G (1999) The $\delta^{3}C$ of tree rings in full-bark and strip-bark bristlecone pine trees in the White Mountains of California. *Global Change Biology*, **5**, 33–40.

Waterhouse JS, Switsur VR, Barker AC *et al*. (2004) Northern European trees show a progressively diminishing response to increasing atmospheric carbon dioxide concentrations. *Quaternary Science Reviews*, **23**, 803–810.

Watmough SA, McNeely R, LaFleure PM (2001) Changes in wood and foliar $\delta^{13}C$ in sugar maple at Gatineau Park, Quebec, Canada. *Global Change Biology*, **7**, 955–960.

Woodward FI (1987) Stomatal numbers are sensitive to increases in CO_2 from preindustrial levels. *Nature*, **327**, 617–618.

Woodward FI (1993) Plant responses to past concentrations of CO_2. *Vegetatio*, **104/105**, 145–155.

Woodward FI, Kelly CK (1995) The influence of CO_2 concentration on stomatal density. *New Phytologist*, **131**, 311–327.

Zimmermann JK, Ehleringer JR (1990) Carbon isotope ratios are correlated with irradiance levels in the Panamanian orchid *Casasetum viriflavum*. *Oecologia*, **83**, 247–249.