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Tracing carbon uptake from a natural CO_2 spring into tree rings: an isotope approach

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Summary We analyzed ^{14}C, ^{13}C and ^{18}O isotope variations over a 50-year period in tree rings of *Quercus ilex* L. trees growing at a natural CO_2 spring in a Mediterranean ecosystem. We compared trees from two sites, one with high and one with low exposure to CO_2 from the spring. The spring CO_2 is free of ^{14}C. Thus, this carbon can be traced in the wood, and the amount originating from the spring calculated. The amount decreased over time, from about 40% in 1950 to 15% at present for the site near the spring, indicating a potential difficulty in the use of natural CO_2 springs for elevated CO_2 research. The reason for the decrease may be decreasing emission from the spring or changes in stand structure, e.g., growth of the canopy into regions with lower concentrations. We used the ^{14}C-calculated CO_2 concentration in the canopy to determine the ^{13}C discrimination of the plants growing under elevated CO_2 by calculating the effective canopy air $^{13}C/^{12}C$ isotopic composition. The trees near the spring showed a 2.5‰ larger ^{13}C discrimination than the more distant trees at the beginning of the investigated period, i.e., for the young trees, but this difference gradually disappeared. Higher discrimination under elevated CO_2 indicated reduced photosynthetic capacity or increased stomatal conductance. The latter assumption is unlikely as inferred from the ^{18}O data, which were insensitive to CO_2 concentration. In conclusion, we found evidence for a downward adjustment of photosynthesis under elevated CO_2 in *Q. ilex* in this dry, nutrient-poor environment.

Keywords: carbon isotope ratio, dendrochronology, elevated carbon dioxide, oxygen isotope ratio, Quercus ilex, radiocarbon analysis.

Introduction

The potential growth response of trees to increased atmospheric CO_2 concentrations is an important factor in the global carbon cycle (Amthor 1995). Forests constitute large reservoirs of carbon, and a change in their carbon storage capacity induced by the fertilizing effect of CO_2 may have an impact on future atmospheric CO_2 concentrations (Dixon et al. 1994). Whereas many studies of the CO_2 concentration effect have been carried out on seedlings, the growth response of mature trees is difficult to assess experimentally because of the long life cycle of trees (Mooney et al. 1991, Körner et al. 1996). One approach has been to study the effects of natural CO_2 springs on intact ecosystems (Miglietta et al. 1993, Grace and van Gardingen 1997). Mineral CO_2 springs are found mainly in active volcanic regions and emit CO_2 at concentrations as high as 100%, thereby raising the atmospheric CO_2 concentration in the immediate vicinity.

A limitation to the value of CO_2 springs for the study of ecosystems under conditions that may prevail in the future is that gases other than CO_2, for example H_2S, may be emitted by the spring with a toxic effect on plants. Further, it may be difficult to find a control site with growth conditions comparable to those at the spring site (Scarascia-Mugnozza et al. 2001). Another important consideration is whether the plants have been exposed to a constant CO_2 concentration throughout their lifetime. In some cases, it is known from historic records that a spring has been active for decades or even centuries. However, the stability over time in the amount of the gas emitted is usually unknown (Etiope and Lombardi 1997). Because CO_2 from the spring is distributed by diffusion and convection to the surrounding area, changes in vegetative cover and canopy height may also influence the concentration of CO_2 reaching the leaves. In particular, stand history and past management have to be considered.

We studied carbon uptake from a CO_2 spring in Toscana, Italy. Previous studies comparing the growth response of *Quercus ilex* L. trees at this site with trees growing under normal CO_2 concentrations have yielded conflicting results. In one study, increased growth of trees during the juvenile period was observed (Hättenschwiler et al. 1997), whereas a second more recent study failed to confirm those findings (Tognetti et al. 2000). We evaluated the use of ^{14}C, ^{13}C and ^{18}O isotopes to determine the effect of the CO_2 spring on these trees. Carbon dioxide is ideally suited as a tracer because CO_2 from the spring is free of ^{14}C and thus has a distinct signal from background atmospheric CO_2. Isotope discrimination in photo-

synthesis was investigated by ^{13}C analysis, which gives an indication of possible changes to water-use efficiency (WUE) at the leaf level due to elevated CO_2 concentration (Farquhar et al. 1982). The discrimination is usually difficult to determine precisely in elevated CO_2 concentration studies because fumigation CO_2 and background air have distinct $^{13}C/^{12}C$ signals. We combined ^{13}C and ^{14}C in a novel way that allowed accurate calculation of the isotope discrimination. Finally, ^{18}O data were used to distinguish more clearly between stomatal conductance and photosynthetic capacity as the driving variable for WUE (Scheidegger et al. 2000).

Methods

Sampling and analysis

The CO_2 spring is located near Lajatico, Italy (43°26′ N 10°42′ E), and is surrounded by a coppiced stand dominated by *Quercus ilex*. Other tree species found at the site include *Quercus pubescens* Willd., *Quercus cerris* L., *Arbutus unedo* L. and *Fraxinus ornus* L. Canopy height of this macchia vegetation is about 5–8 m. The climate is Mediterranean, with cool, wet winters and dry, hot summers. A full description of the site is given in Tognetti et al. (2000). The spring emits almost pure CO_2 and increases CO_2 concentrations over an area of approximately 0.7 ha. *Quercus ilex* trees near the spring ("high-CO_2 site") were exposed to about 700 μmol mol^{-1} CO_2 as determined by infrared gas analyzers and absorptive diffusion tubes. Significant short-term variations in CO_2 occur depending on weather conditions, in particular wind speed, but the CO_2 gradient with height in the canopy is reported to be small (Tognetti et al. 2000). A second site was chosen roughly 150 m from the CO_2 spring ("low-CO_2 site") at a slightly lower elevation. The same site was selected as a control by Tognetti et al. (2000), but it turned out to be exposed to a small amount of CO_2 from the spring (see below). Growth conditions were similar at the low- and high-CO_2 sites concerning slope aspect, soil type, and water and nutrient availability (Raiesi Gahrooee 1998).

In the experimental area, coppicing takes place every 40–50 years (Hättenschwiler et al. 1997). Based on tree-ring age, the trees in the stand germinated or resprouted mainly between 1940 and 1960 (Table 1). Therefore, it is likely that the trees were coppiced shortly before 1940. Single erect stems were selected, but it was impossible to distinguish between trees originating from seedlings and from sprouts. No positive abrupt growth change, i.e., growth release after suppression, occurred after 1940 (Tognetti et al. 2000), so any major stand disturbances in the investigated period (1951–1998) can probably be excluded. Tree stem disks were collected in 1998. Wood samples from three *Q. ilex* trees in each of the low- and high-CO_2 sites were used for isotope analysis, the trees being a subset of those sampled in a previous tree-ring growth study (Tognetti et al. 2000). Dating of the tree rings was possible, but it was hindered by the presence of density fluctuations (false rings) common in this climate (Cherubini et al. 2003). Disks were split into 10-year intervals (1951–1960, 1961–1970, 1971–1980, 1981–1990, 1991–1998).

Table 1. The approximate age structure of the low- and the high-CO_2 sites. Shown is the number of *Q. ilex* trees with the innermost ring dating from a given year (Tognetti et al. 2000). Numbers in bold face indicate trees that were used for isotope analysis. Because the cores and cross sections were taken at a height of 1 m rather than at the stem base, the germination date is about 2–5 years earlier than the date of the innermost ring.

Date of innermost ring	Low-CO_2 site	High-CO_2 site
1933	1	
1942		1
1945		2
1946	**1**/2	
1949	**1**	
1950		**2**
1951		1/**1**
1954	**1**	
1957	1	1
1958		1

Samples were milled in a centrifugal mill (Retsch, Germany). The $^{13}C/^{12}C$ ratio was determined by combustion of the wood powder to CO_2 in an elemental analyzer followed by analysis in an isotope-ratio mass spectrometer (Delta S, Finnigan MAT, Bremen, Germany). The $^{18}O/^{16}O$ ratio was determined by pyrolysis to CO (Saurer et al. 1998). The isotope ratios are given in the δ-notation relative to international standards:

$$\delta^{13}C = 1000\left(\frac{^{13}C/^{12}C_{sample}}{^{13}C/^{12}C_{PDB}} - 1\right)$$

and

$$\delta^{18}O = 1000\left(\frac{^{18}O/^{16}O_{sample}}{^{18}O/^{16}O_{VSMOW}} - 1\right)$$

To sample atmospheric CO_2, air was pumped through Teflon tubes from different heights into evacuated stainless steel containers (1.5 l in volume) on May 2, 1999. The same apparatus was used to sample spring CO_2 with a tube held directly at the vent. The bottled CO_2 was cryogenically purified in a vacuum extraction line and analyzed with the dual inlet system of the Delta S. For the ^{14}C-analysis, samples from different trees were pooled for each 10-year period. The ^{14}C-content of the wood samples was measured by accelerator mass spectrometry at the PSI/ETH facility in Zurich, Switzerland. Values of $\Delta^{14}C$ are given as relative deviations of the ^{14}C activity of the sample from the Oxalic Acid I standard in ‰ after accounting for ^{14}C fractionation with a $\delta^{13}C$ correction. Statistics for the significance of linear regressions were assessed by Student's *t*-test.

Calculations

Fraction of carbon in the wood originating from the spring
The CO_2 assimilated by the trees is either spring CO_2 or background (atmospheric) CO_2. Therefore, the following mass balance equation applies to the fraction x of carbon in the wood originating from the spring:

$$\Delta^{14}C_{tree} = x\Delta^{14}C_{spring} + (1 - x)\Delta^{14}C_{atm} \quad (1)$$

where $\Delta^{14}C_{tree}$, $\Delta^{14}C_{spring}$ and $\Delta^{14}C_{atm}$ are the $\Delta^{14}C$ values of tree rings, spring CO_2 and atmospheric CO_2, respectively. Rearranging Equation 1 gives:

$$x = \frac{\Delta^{14}C_{tree} - \Delta^{14}C_{atm}}{\Delta^{14}C_{spring} - \Delta^{14}C_{atm}} \quad (2)$$

Percentage values are obtained by multiplying by 100. Equation 2 holds for any time in the past provided that the corresponding $\Delta^{14}C$ values are used.

CO_2 concentration in the canopy The average CO_2 concentration reaching the canopy (c_{canopy}) can be calculated from the ^{14}C data according to van Gardingen et al. (1995). In the derivation shown here, which is more general, we do not assume that control trees reflect background ^{14}C, nor do we assume that the source is free of ^{14}C. Because the total CO_2 concentration in the canopy has a contribution from the background air (c_{atm}) and from the spring (c_{spring}), we set:

$$c_{canopy} = c_{atm} + c_{spring} \quad (3)$$

Now, it is convenient to introduce the enrichment factor f of the CO_2 concentration above ambient (because c_{atm} was not constant during the investigated period):

$$c_{canopy} = fc_{atm} \quad (4)$$

As an example, a value of $x = 0.5$ (50%) would mean that the trees received half of their carbon from the spring and half from the background air. This would correspond to a doubling of the CO_2 concentration, i.e., $f = 2$, which can be calculated from:

$$x = \frac{c_{canopy} - c_{atm}}{c_{canopy}} = 1 - \frac{c_{atm}}{c_{canopy}} = 1 - \frac{1}{f} \quad (5)$$

and thus:

$$f = \frac{1}{1 - x} = \frac{1}{1 - \dfrac{\Delta^{14}C_{tree} - \Delta^{14}C_{atm}}{\Delta^{14}C_{spring} - \Delta^{14}C_{atm}}} \quad (6)$$

Data for $\Delta^{14}C_{atm}$ for recent decades can be found in the literature (Levin et al. 1994, Levin and Kromer 1997). When the atmospheric CO_2 concentrations are considered (data from Keeling and Whorf 2001), c_{canopy} can be calculated from Equation 4.

$\delta^{13}C$ of canopy air The canopy air $\delta^{13}C$ is influenced by the addition of spring CO_2 in varying amounts. A two-member mixing model can be applied (Keeling 1958, see also results), neglecting possible influences of photosynthesis and respiration:

$$c_{canopy}\delta^{13}C_{canopy} = c_{atm}\delta^{13}C_{atm} + c_{spring}\delta^{13}C_{spring} \quad (7)$$

We now make use of the ^{14}C data, which give us independent information on c_{spring} and c_{canopy}, and thus enable us to resolve Equation 7 (because the concentrations are eliminated). Using $c_{atm}/c_{canopy} = 1 - x$ and $c_{spring}/c_{canopy} = x$ (from Equations 3 and 5), we get:

$$\delta^{13}C_{canopy} = \delta^{13}C_{atm} + x\left(\delta^{13}C_{spring} - \delta^{13}C_{atm}\right)$$

and finally by replacing x (Equation 2):

$$\delta^{13}C_{canopy} = \delta^{13}C_{atm} + \frac{\Delta^{14}C_{tree} - \Delta^{14}C_{atm}}{\Delta^{14}C_{spring} - \Delta^{14}C_{atm}}\left(\delta^{13}C_{spring} - \delta^{13}C_{atm}\right) \quad (8)$$

To calculate $\delta^{13}C_{canopy}$, we thus need the ^{14}C composition of the tree rings plus the dual carbon isotope information (^{14}C and ^{13}C) from the spring as well as from the atmospheric CO_2. As for $\Delta^{14}C_{atm}$, values of $\delta^{13}C_{atm}$ for the past can be found in the literature (Friedli et al. 1986, Keeling et al. 1989). The other parameters in Equation 8 were measured.

Carbon isotope discrimination The results from Equation 8 ($\delta^{13}C_{canopy}$) in combination with the $\delta^{13}C$ values of the tree rings can be used to determine the isotope discrimination by the trees (Farquhar et al. 1982), defined as positive numbers:

$$\Delta^{13}C = \frac{\delta^{13}C_{canopy} - \delta^{13}C_{tree}}{1 + \delta^{13}C_{tree}/1000} \quad (9)$$

Results

Carbon-14

The $\Delta^{14}C$ values of trees growing near the CO_2 spring were consistently lower than the values of trees from the more distant site, indicating the uptake of a large amount of "dead" (^{14}C-free) CO_2 near the spring (Table 2). There were, however, significant variations at both sites in the last 50 years that did not reflect spring uptake. The ^{14}C content of atmospheric CO_2 almost doubled in the 1960s as a result of nuclear bomb tests (corresponding to 1000‰ $\Delta^{14}C$, see Figure 1). For the quantitative evaluation of carbon uptake from spring CO_2, we calculated averages of the atmospheric ^{14}C concentration ($\Delta^{14}C_{atm}$) for the same periods as were analyzed for the tree rings. The averages are shown in Figure 1, together with corresponding

Table 2. Values of $\Delta^{14}C$ and $\delta^{13}C$ of wood from *Q. ilex* trees growing in Lajatico (low- and high-CO_2 sites), calculated parameters (enrichment factor *f*, CO_2 concentration in the canopy (c_{canopy}) and $\delta^{13}C_{canopy}$) and data for the background atmosphere taken from the literature (c_{atm} and $\delta^{13}C_{atm}$). See text for details. The standard deviation (SD) for $\Delta^{14}C$ is the analytical uncertainty, whereas SD for $\delta^{13}C$ indicates the variation between the trees.

		1951–1960	1961–1970	1971–1980	1981–1990	1991–1998
$\Delta^{14}C \pm SD$ (‰)	Low CO_2	–1.1 ± 5.9	457.9 ± 7.7	279.0 ± 6.9	155.5 ± 6.5	81.9 ± 6.3
	High CO_2	–301.1 ± 5.0	–97.0 ± 5.9	–6.7 ± 6.1	–33.1 ± 5.8	–24.1 ± 5.9
$\delta^{13}C \pm SD$ (‰)	Low CO_2	–25.38 ± 0.27	–25.54 ± 0.49	–25.33 ± 0.51	–25.23 ± 0.32	–25.87 ± 0.16
	High CO_2	–28.38 ± 1.31	–27.35 ± 1.09	–26.68 ± 0.43	–26.24 ± 0.72	–25.94 ± 0.49
f	Low CO_2	1.09	1.10	1.06	1.03	1.03
	High CO_2	1.56	1.77	1.36	1.23	1.15
c_{atm} (ppm)	Background	314.2	321.0	332.0	346.7	360.2
c_{canopy} (ppm)	Low CO_2	342.8	352.4	351.8	358.3	372.1
	High CO_2	490.0	569.0	453.0	428.2	412.5
$\delta^{13}C_{atm}$ (‰)	Background	–6.83	–7.01	–7.39	–7.69	–7.93
$\delta^{13}C_{canopy}$ (‰)	Low CO_2	–7.02	–7.20	–7.49	–7.74	–7.97
	High CO_2	–7.64	–7.92	–7.85	–7.96	–8.08

tree ring values for the two study sites. The lower the tree ring ^{14}C values are relative to atmospheric CO_2, the higher the contribution from the spring. The fraction of carbon in the wood originating from the spring is calculated with Equation 2, whereby the ^{14}C content of the spring CO_2 is assumed to be zero (i.e., $\Delta^{14}C_{spring} = -1000‰$).

The results in Figure 2 show that *x* decreased over time. The average for the trees at the site near the spring was 39.7 ± 5.5% for the 1950s and 1960s, gradually decreasing to less than 15% in the 1990s. Uptake of spring CO_2 at the low-CO_2 site decreased over time from a maximum value of 9% at the beginning of the investigated period to about 3% at present. The values for the sixties might be less precise than for the other decades because of the steep gradient in $\Delta^{14}C_{atm}$, although it has been shown that tree rings faithfully record even short-term changes in $\Delta^{14}C_{atm}$ (Grootes et al. 1989). There is, in fact, a high correlation ($r^2 = 0.98$) between measured data ($\Delta^{14}C_{tree}$) and literature data ($\Delta^{14}C_{atm}$) for the low-CO_2 site, indicating the reliability of the radiocarbon tree-ring data even in the absence of replicates (because samples from different trees were pooled for the ^{14}C analysis).

The *f* values indicating the CO_2 enrichment above ambient (Equation 6) generally decreased over time (in the same way as the *x* values in Figure 2), ranging between 1.03 and 1.10 at the low-CO_2 site and between 1.15 and 1.77 at the high-CO_2 site. The corresponding c_{canopy} values are shown in Table 2.

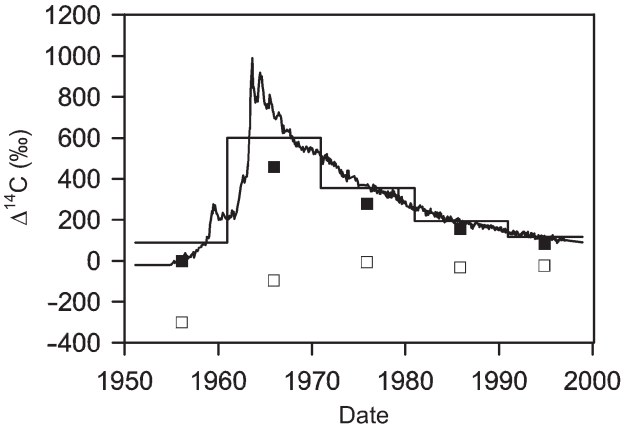

Figure 1. Tree ring $\Delta^{14}C$ values from the (■) low- and (□) high-CO_2 sites. The thin line shows the ^{14}C activity of atmospheric CO_2 using a combined data set from Vermunt (Austria; Levin et al. 1994) and from Schauinsland (Germany; Levin and Kromer 1997). Averages of these data for the periods 1951–1960, 1961–1970, 1971–1980, 1981–1990 and 1991–1998 are also indicated.

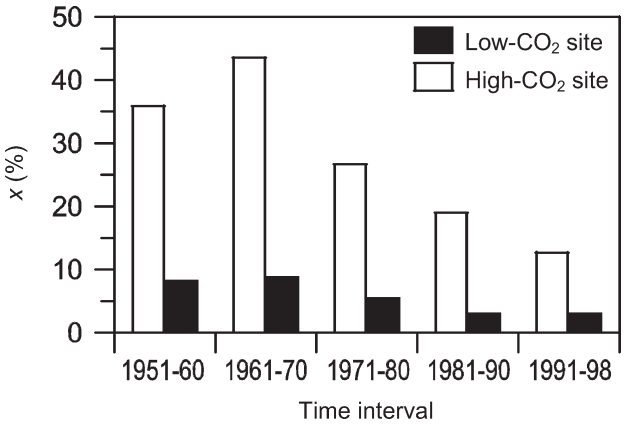

Figure 2. The percentage of carbon in trees originating from the CO_2 spring (*x*). See text for details.

Carbon-13

The $\delta^{13}C$ value of pure spring CO_2 was –9.09 ± 0.1‰. This value is similar to that of background air, which is about –8‰ at present. In order to determine precisely the ^{13}C discrimination by the trees, we needed the canopy air $\delta^{13}C$. A gradient in $\delta^{13}C$ values was observed in atmospheric samples with different mixing ratios of spring CO_2 and background air (Figure 3). Sampling was done on a sunny, slightly windy day (May 2, 1999), at different distances around the two sites. The samples collected at 2 to 8 m above ground did not show a trend with height ($r^2 = 0.002$). Data in Figure 3 are shown as a "Keeling-plot," where the relationship between $[CO_2]^{-1}$ and $\delta^{13}C$ is plotted (Keeling 1958). A linear relationship is apparent and the *y*-intercept corresponds to the direct measurement of the spring $\delta^{13}C$ value. This shows that $\delta^{13}C_{canopy}$ can be expressed reasonably well by the mixing model described in Equations 7 and 8. The decrease in atmospheric $\delta^{13}C$ due to the combustion of fossil carbon was also considered. The respective values for the investigated time period are shown in Table 2. After inserting all required ^{14}C and ^{13}C data into Equation 8, the estimated $\delta^{13}C_{canopy}$ values varied between –7.64 and –8.08‰ at the high-CO_2 site and between –7.02 and –7.92‰ at the low-CO_2 site (Table 2). The difference between $\delta^{13}C_{canopy}$ and $\delta^{13}C_{atm}$ never exceeded 0.91‰.

The carbon isotope discrimination was calculated according to Equation 9. Tree ring $\Delta^{13}C$ values from the low-CO_2 site varied slightly around 18.5‰ (Figure 4), although there was a small temporal trend of –0.18‰ per decade. In contrast, the discrimination for trees at the spring site was much larger at the beginning of the investigated period, being 2.5‰ greater than at the low-CO_2 site during 1951–1960. Discrimination then gradually decreased until the difference between the sites disappeared completely for the 1991–1998 period (Figure 4). Variations in ^{13}C discrimination can be largely attributed to increased concentrations of CO_2 near the spring. From the regression analysis (with individual trees, including low- and high-CO_2 sites), a change of 100 ppm CO_2 concentration resulted in a 1.0‰ increase in the ^{13}C discrimination ($r^2 = 0.40$; $P < 0.001$).

The discrimination is caused by isotope fractionation during the diffusion of CO_2 through the stomata ($a = 4.4$‰) and CO_2 fixation by the enzyme Rubisco ($b = 27$‰) according to the following formula:

$$\Delta^{13}C = a + (b - a)\frac{c_i}{c_a} \quad (10)$$

where c_i/c_a is the ratio of intercellular to ambient CO_2 concentrations (Farquhar et al. 1982). This ratio (and thus $\Delta^{13}C$) is determined by the balance of stomatal conductance and photosynthetic capacity, and can be considered as a set point for the integration and coordination of gas exchange in response to a changing environment (Ehleringer and Cerling 1995). High values of c_i/c_a for *Q. ilex* in an elevated CO_2 concentration inferred from Equation 10 indicate a relatively weak limitation of photosynthesis by diffusion.

Oxygen-18

Although the carbon isotope ratio alone does not allow clear differentiation between variations in c_i/c_a caused by changes in stomatal conductance and photosynthetic capacity, the inclusion of $\delta^{18}O$ may help to make this distinction (Scheidegger et al. 2000). In the leaves of transpiring plants, a significant ^{18}O enrichment relative to the source water takes place, as the lighter water molecules evaporate more easily from leaf pores. Relative humidity determines the degree of enrichment possible (Dongmann et al. 1974), but high transpiration rates tend to reduce this enrichment (at a given relative humidity) through "flushing" of the leaves with light source water (Farquhar and Lloyd 1993). The leaf water signal is transferred to the organic matter by isotope exchange reactions (Sternberg et al. 1986). When comparing plants growing close together but subject to a treatment effect (e.g., CO_2 fumigation), source water $\delta^{18}O$ and weather are identical for all plants, so differences in $\delta^{18}O$ of organic matter are most likely caused by differing transpira-

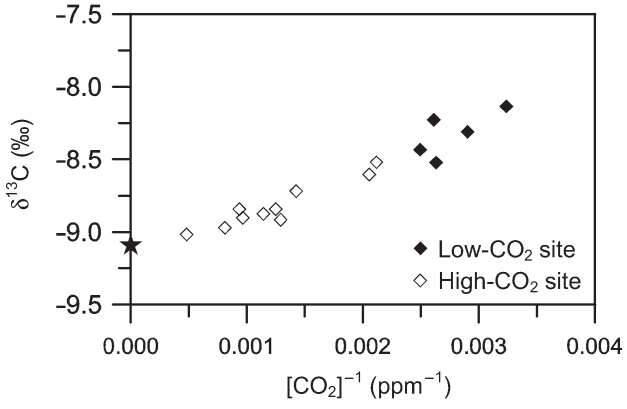

Figure 3. Values of $\delta^{13}C$ of recent atmospheric samples collected at different distances around the CO_2 spring, shown as a "Keeling-plot." The star indicates the measured $\delta^{13}C$ value of pure CO_2 collected directly from the spring (–9.1‰).

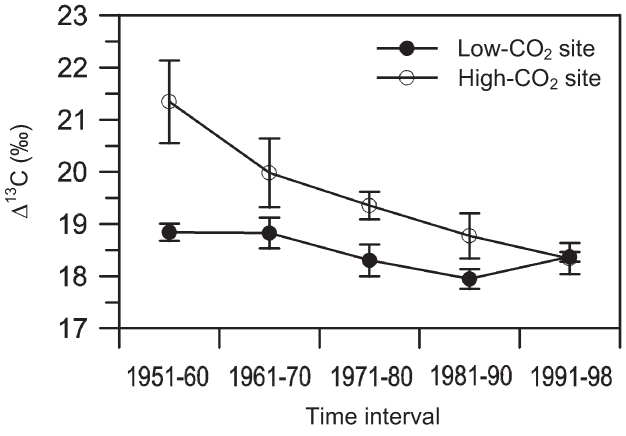

Figure 4. The tree-ring $\Delta^{13}C$ values from the low- (●) and high-CO_2 (○) sites (with standard error).

tion rates or stomatal conductance (because these are the only factors affecting oxygen isotope fractionation that can differ among treatments). The data shown in Figure 5 indicate a minor influence of CO_2 concentrations on $\delta^{18}O$. Because the relationship between $\Delta^{13}C$ and CO_2 is much stronger ($r^2 = 0.40$; $P < 0.001$) than the relationship between $\delta^{18}O$ and CO_2 ($r^2 = 0.13$; $P < 0.047$), the inferred changes in c_i/c_a are probably caused by a response in the photosynthetic capacity and not in stomatal conductance.

Discussion

Our data constitute a test for the usefulness of natural CO_2 springs in studying future greenhouse conditions with respect to tree growth. Carbon dioxide concentrations in vegetation surrounding CO_2 springs are not as stable as concentrations in vegetation in a FACE facility (Hendrey et al. 1999). Variations due to wind conditions result in large short-term fluctuations, which would not be of great concern if the long-term averages were stable. Our ^{14}C data, however, indicate that this may not always be the case. Values of ^{14}C in the surroundings of CO_2 springs have been used to measure the uptake of "dead" CO_2 in leaves (Bruns et al. 1980). It was shown that the effective CO_2 concentration over the life of a leaf could be estimated (van Gardingen et al. 1995). We demonstrated that ^{14}C in tree rings could be used to estimate canopy CO_2 concentration over long periods (50 years). Surprisingly, we found a strongly decreasing trend in the amount of carbon from the spring in the tree rings, and thus the canopy CO_2 concentrations must have decreased over time as well. The most likely explanations for the temporal trend are a decrease in the source strength or a concentration gradient with height. In both cases, the trees would have been exposed to higher CO_2 concentrations when they were young. Further, the practice of coppicing in this area may have influenced our results. Based on the age structure of the stand (Tognetti et al. 2000), trees were coppiced shortly before 1950 and not in the analyzed period. Therefore, a disturbing influence of stand management on our results is unlikely.

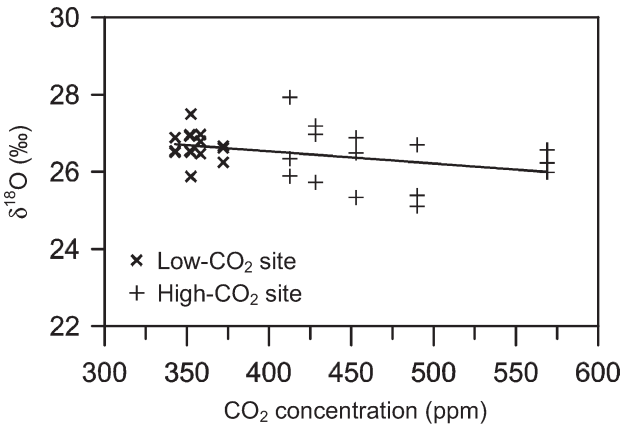

Figure 5. Values of $\delta^{18}O$ of *Quercus ilex* trees as a function of the ^{14}C-calculated CO_2 concentration ($r^2 = 0.13$).

Higher canopy CO_2 concentrations in the 1950s and 1960s might be a reason for the increased growth of young *Q. ilex* trees found at the Lajatico site at elevated CO_2 concentration compared with control trees (Hättenschwiler et al. 1997), although a different set of trees was used in that study.

In artificial fumigation systems, $\delta^{13}C$ is often negative (around –30‰ when CO_2 is obtained from petrochemical production or combustion of organic material) and thus can be used as a tracer for the carbon flux in different ecosystem compartments, such as soil fractions (Leavitt et al. 1994). However, when such an isotopically depleted gas is used for elevated CO_2 concentration studies, plant isotope discrimination is hard to determine (Picon et al. 1996). In principal, the discrimination can be calculated from the $\delta^{13}C$ of the canopy air, which can be estimated either from continuous CO_2 measurements or from $\delta^{13}C$ values of C_4 plants grown in the same environment (Marino and McElroy 1991). Yet, it is difficult to measure the average effective $\delta^{13}C$ composition of air (i.e., the average during the growing period of the plant tissue investigated) with high precision (~0.2‰) because of the large isotope difference between background CO_2 and fumigation CO_2, and because of inevitable fluctuations in CO_2 concentration in the canopy. Further, C_4 plants change their discrimination in response to environmental conditions (Buchmann et al. 1996) and a modification could occur as a result of CO_2 fumigation. Thus, there is the risk of finding artifactual "CO_2 effects" when the $\delta^{13}C_{canopy}$ is inadequately determined. Indeed, few data on discrimination changes in response to increased CO_2 concentrations have been published (Williams et al. 2001). The ^{13}C discrimination, however, is a sensitive indicator of changes in physiological parameters, because of its relation to c_i/c_a and WUE. Stable isotope analysis may therefore reveal whether plants react more strongly through stomatal conductance or photosynthetic capacity to increasing CO_2 concentration. We developed a simple equation that uses the ^{14}C-derived CO_2 concentration in the canopy to calculate the ^{13}C discrimination (Equation 8). The correction term for spring CO_2 in Equation 8 is most significant when the difference between $\delta^{13}C_{spring}$ and $\delta^{13}C_{atm}$ is large (the difference is rather small for the spring in Lajatico). Nevertheless, even for small differences the correction is important because changes in WUE and accordingly in discrimination may also be rather small. Equation 8 is applicable to artificial fumigation systems when petrochemical CO_2 is used, which is ^{14}C free (Leavitt et al. 1994). However, when CO_2 originating from the combustion of organic matter is used, the difference in the denominator of the correction term ($\Delta^{14}C_{source} - \Delta^{14}C_{atm}$) is too small. The advantage of our approach over monitoring $\delta^{13}C_{atm}$ with C_4 plants is that the analysis can be done *a posteriori*, i.e., without continuous records for $\delta^{13}C_{canopy}$, as in tree-ring studies, or in experiments where C_4 plants were not grown.

We found increased ^{13}C discrimination in *Q. ilex* trees at higher CO_2 concentrations. A similar result was found at another Italian mineral spring (Miglietta et al. 1998). We observed the greatest increase in $\Delta^{13}C$ when the trees were young, but this was also the time of highest atmospheric CO_2

enrichment in the canopy. Therefore, the influences of age and CO_2 concentration cannot be evaluated separately. According to the model devised by Farquhar, greater discrimination can be caused by increased stomatal conductance or reduced photosynthetic capacity (Farquhar et al. 1989). In response to elevated CO_2 concentration, stomatal conductance is expected to decrease or remain unchanged (Woodward 1987, Tognetti and Johnson 1999). Our $\delta^{18}O$ results indicate a limited response of stomatal conductance to elevated CO_2 concentration, implying that the trees do not reduce transpiration rates in response to elevated CO_2 concentration. The absence of a stomatal response might also be due to seasonal differences in water use that are hidden in the bulk tree ring analysis. For instance, faster use of soil water at the low-CO_2 site (because of higher conductance) could result in higher drought stress later in the growing season (and subsequent stomatal closure). These effects could offset each other to yield a negligible net effect on tree ring ^{18}O. We assume, however, that the reason for the increased discrimination is the down-regulation of photosynthesis and lower photosynthetic capacity. Several mechanisms have been invoked to explain the CO_2 acclimation processes, particularly changes in sink strength and nutrient limitation (Murray et al. 2000). Miglietta et al. (1998) emphasized that long-term adjustment of photosynthesis was likely to occur on nutrient-poor soils, although the adjustment may depend on the species. This agrees with our results from Lajatico, which is a nutrient-poor site (Raiesi Gahrooee 1998). Our results are consistent with an examination of the isotopic discrimination in *Erica arborea* L. trees along a nitrogen gradient in the vicinity of a CO_2 vent in Italy (Bettarini et al. 1995). According to that study, nitrogen availability had a major effect on leaf N and photosynthetic capacity, and consequently on c_i. Discrimination increased in response to elevated CO_2 concentration only when soil nitrogen was limiting. At two other CO_2 springs in Italy, no down-regulation of photosynthesis in *Q. pubescens* was found early in the growing season (Stylinski et al. 2000), when sink strength is high. However, the authors speculated that this might not be the case in summer and autumn on account of the reduced sink demand. Our results for *Q. ilex* suggest that the trees were unable to profit from elevated CO_2 concentration because of a reduction in photosynthetic capacity associated with limited soil nitrogen availability. This conclusion is consistent with the finding by Tognetti et al. (2000) concerning unchanged tree-ring growth at the site.

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