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Homeostatic gas-exchange parameters inferred from $^{13}C/^{12}C$ in tree rings of conifers

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Abstract The CO_2 concentration of the atmosphere has increased by almost 30% in the past two centuries, with most of the increase (> 5 Pa) during the past 60 years. Controlled environment studies of crop plants dependent on the C_3 photosynthetic pathway indicate that an increase of this magnitude would enhance net photosynthesis, reduce stomatal conductance, and increase the difference in CO_2 concentration across the stomata, i.e., CO_2 concentration outside the leaf to that within (c_a–c_i). Here we report evidence, based on stable isotope composition of tree rings from three species of field-grown, native conifer trees, that the trees have indeed responded. However, rather than increasing c_a–c_i, intercellular CO_2 concentrations have shifted upward to match the rise in atmospheric concentrations, holding c_a–c_i constant. No differences were detected among Douglas-fir (*Pseudotsuga menziesii*), ponderosa pine (*Pinus ponderosa*), or western white pine (*Pinus monticola*). The values of c_a–c_i were inferred from stable carbon isotope ratio ($\delta^{13}C$) of tree ring holocellulose adjusted for the 0.6–2.6‰ difference between holocellulose and whole sapwood. The cellulose extraction removed contaminants deposited in the tree ring after it formed and the adjustment corrected for the enrichment of cellulose relative to whole tissue. The whole sapwood values were then adjusted for published estimates of past atmospheric $\delta^{13}CO_2$ and CO_2 concentrations. To avoid confounding tree age with CO_2, cellulose deposited by saplings in the 1980s was compared to cellulose deposited in the inner rings of mature trees when the mature trees were saplings, between 1910–1929 and 1941–1970; thus saplings were compared to saplings. In a separate analysis, the juvenile effect, which describes the tendency for $\delta^{13}C$ to increase in the first decades of a tree's life, was quantified independent of source CO_2 effects. This study provides evidence that conifers have undergone adjustments in the intercellular CO_2 concentration that have maintained c_a–c_i constant. Based on these results and others, we suggest that c_a–c_i, which has also been referred to as the intrinsic water-use efficiency, should be considered a homeostatic gas-exchange set point for these conifer species.

Key words *Pseudotsuga menziesii* · *Pinus ponderosa* · *Pinus monticola* · Carbon isotope discrimination · Water-use efficiency

Introduction

Because CO_2 is the sole atmospheric substrate for photosynthesis by terrestrial plants, fluctuations in concentrations of CO_2 [CO_2] in the atmosphere strongly influence plant function. Two dramatic post-glacial increases have been described; the first at the end of the last glacial, approximately 12,000 years ago, when concentrations increased from about 200 ppm to 280 ppm (Neftel et al. 1982; Barnola et al. 1987), and the second during the last 200 years, when concentrations increased from 280 ppm to 360 ppm (Friedli et al. 1986; Keeling et al. 1989). Responses are obvious when plants are exposed to high concentrations for short periods in controlled environments (Kramer 1981; Eamus and Jarvis 1989) and agricultural crop plants consistently show positive growth responses even in the field (Pitelka 1994). However, long-term data from natural forest trees remains scarce, partly because of the difficulty of controlled experimentation on large trees (Mooney et al. 1991; Pitelka 1994).

Nonetheless, several lines of circumstantial evidence indicate that a change in [CO_2] affects forest trees. First,

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CO_2-induced growth increases have been detected with dendrochronology (LaMarche et al. 1984; Graybill and Idso 1993); however, other studies have found no such effect (Graumlich 1991) or effects too large to be attributable to [CO_2] alone (Kienast and Luxmoore 1988). Second, a comparison of the stomatal densities of modern leaves to those of preindustrial herbarium-specimens provided clear evidence of morphological (and presumably physiological) responses to atmospheric [CO_2] in mature trees (Woodward 1987). Woodward's analyses were accompanied by controlled environment studies that showed that variation in CO_2 mole fraction (Woodward 1987) and partial pressure (Woodward and Bazzaz 1988) could induce similar shifts.

A third line of evidence, based on the ratio of the stable isotopes of carbon ($\delta^{13}C$) in plant tissue, also indicates that physiological change in response to atmospheric [CO_2] has occurred in woody plants. Isotopic composition of plant tissue is determined in part by the isotopic composition of CO_2 in the atmosphere and in part by the extent to which plants discriminate against the heavy isotope in photosynthesis. Correcting for source air effects, one can focus on photosynthetic discrimination (Δ) by the plant (Farquhar and Richards 1984). Discrimination by plants dependent on the C_3 photosynthetic pathway is linearly related to the ratio of the [CO_2] within the leaf (c_i) divided by the [CO_2] outside the leaf (c_a) (Farquhar et al. 1982). Polley et al. (1993) detected shifts in gas exchange but not in stable carbon isotope discrimination of crop plants grown in controlled environments across the range of [CO_2] since full glacial. Similar studies of fossil leaves have found evidence of shifts in both stomatal density and isotopic discrimination in *Pinus flexilis* (Van de Water et al. 1994) and *Salix herbacea* (Beerling et al. 1993) since full glacial. Moreover, Beerling et al. (1993) observed upward shifts in isotopic discrimination of *S. herbaceae* during the last two centuries.

Such shifts in discrimination and in c_i/c_a, which has been considered a gas-exchange set point (Ehleringer 1993), highlight the need to identify homeostatic parameters, if such things exist. This identification is particularly important because plants cannot maintain all gas exchange variables constant as [CO_2] change. For example, if net photosynthesis (A) is described as:

$$A = g_c * (c_a - c_i) \qquad (1)$$

where g_c is the leaf conductance to CO_2, c_a is the ambient [CO_2] and c_i is the internal concentration at the base of the stomata, predicting the influence of a change in c_a on A would depend on the ability to predict changes in c_i and g_c. Some experimental evidence indicates that plants maintain the ratio c_i/c_a constant as ambient [CO_2] increases (Wong et al. 1979; Masle et al. 1990; Polley et al. 1993). If [CO_2] have risen from 280 ppm to 360 ppm since the industrial revolution and if c_i/c_a were maintained at a constant 0.75, c_i would have increased from 210 ppm to 270 ppm. The difference in [CO_2] between the ambient air and the leaf interior (c_a–c_i) would have increased from 70 to 90 ppm. Were conductance held constant, photosynthetic rates would have increased by 90/70, or 29%. Other evidence suggests that c_i may be held constant across a range of c_a (Francey and Farquhar 1982; Ehleringer and Cerling 1995). If c_i were maintained at 210 ppm and conductance were held constant, net photosynthetic rates would have increased by 150/70, or 214%. And lastly, if c_a–c_i and stomatal conductance were held constant, then photosynthetic rates would not have changed. These examples demonstrate that homeostatic maintenance of one variable requires substantial adjustment of other variables.

Isotopic ratios of carbon can be used to reconstruct past ratios of intercellular to external CO_2 concentrations (Van de Water et al. 1994). However, other sources of variation in isotopic composition, such as variation in $\delta^{13}C$ of source air, must either be removed or quantified. The importance of correcting for source air effects was noted above. Second, analysis of extracted cellulose minimizes variation due to chemical composition (Park and Epstein 1961), but results in enrichment of about 1.5‰ relative to whole tissue (Schleser 1990). Third, isotope ratios frequently shift as trees age or grow larger (Craig 1954; Francey and Farquhar 1982; Yoder et al. 1994). Fourth, isotopic ratios are modified deep in the crown due to shade-induced increases in c_i/c_a (Francey and Farquhar 1982; Ehleringer et al. 1986) and soil respiration-induced shifts in $\delta^{13}C$ of source air (Medina and Minchin 1980; Sternberg et al. 1989; Broadmeadow et al. 1992; Broadmeadow and Griffiths 1993).

In this study we analyzed the carbon isotope ratios of tree ring cellulose deposited over the last 80 years in trees of ponderosa pine (*Pinus ponderosa* Laws.), Douglas-fir (*Pseudotsuga menziesii* [Mirb] Franco var. *glauca*) and western white pine (*Pinus monticola* Dougl.) growing naturally at the Priest River Experimental Forest (PREF) in northern Idaho, United States. Isotopic discrimination was calculated by assuming that the carbon isotope ratio of past atmospheric [CO_2] could be reconstructed from ice core data. The discrimination data were then combined with [CO_2] that were similarly reconstructed from ice core data. This combination of data provided an algebraic means of estimating c_i, c_i/c_a, and c_a–c_i to determine which variable, if any, has been held constant over the course of the twentieth century.

Materials and methods

Sampling

Ponderosa pine, western white pine, and Douglas-fir trees were sampled at the PREF in northern Idaho, United States (116°50'W, 48°21'N), in the Selkirk Range of the Rocky Mountains. No tree was more than 3 km from any other, nor were trees chosen if they showed any sign of insect or disease attack. All were at altitudes between 800 and 950 m. All trees sampled in 1988 and 1989 were mature dominants or codominants and all bore mature cones in the year of sampling. Young saplings and trees of intermediate age were sampled in 1991. Each of the mature and intermediate trees was felled and two disks were cut from the bole: one near the base and one near the top of the stem. From the basal disk two wood samples, each representing a single annual growth ring, were then removed. One sample represented the trees as saplings and one represented the recently formed wood near the base. From the upper-crown disk one sample was removed to represent recent wood from near the top of the trees. A single sample was collected from each of the saplings, at 2 m above the ground, to represent recent wood in young trees. Each sample was carefully dated and divided into earlywood and latewood based on distinct changes in color and resistance to peeling with a sharp blade. This sampling regime allowed us to account for potential changes in isotopic composition due to the combined effects of refixation of CO_2 respired from the soil (Medina and Minchin 1980; Sternberg et al. 1989; Broadmeadow et al. 1992) and lower *A*/*g* of shade leaves near the bottom of the canopy (Francey and Farquhar 1982; Ehleringer et al. 1986).

A preliminary experiment was conducted to determine whether it was necessary to extract holocellulose for determination of $\delta^{13}C$ rather than simply using the whole wood. Earlier workers have noted that changes in tissue composition strongly influence the $\delta^{13}C$ of whole wood due to differences in fractionation among biosynthetic pathways (Park and Epstein 1961). Although the published literature leaves little doubt that holocellulose differs from whole wood (Leavitt and Long 1986), we hypothesized that if the difference were constant the cellulose extraction might be unnecessary. We compared the quantity $\delta^{13}C_{\mathrm{cellulose}} - \delta^{13}C_{\mathrm{wood}}$, which we will refer to as the "offset", among samples chosen to maximize the likelihood of differences. A three-way factorial design was used to analyze variation among the three species, earlywood versus latewood, and sapwood versus heartwood. Holocellulose was extracted according to the procedures of Leavitt and Danzer (1993). The results, presented in Table 1, led to the conclusion that earlywood differed from latewood and that the difference between heartwood and sapwood varied among species. Mean offsets by tissue type are presented in Table 2. The many sources of variability in the offset, and their interactions, led us to conclude that cellulose extractions were indeed necessary. All isotopic data presented below are therefore from extracted holocellulose. In addition, we reduced experimental error by separately analyzing earlywood and latewood.

Table 1 Results of analysis of variance of stable carbon isotope composition ($\delta^{13}C$) of cellulose and whole wood, which was collected from earlywood and latewood, which were in turn collected from sapwood and heartwood of three conifer species. Error *df* = 43

Source of variation	*F*	*P* > *F*
Species	1.53	0.23
Sapwood/heartwood	1.90	0.18
Species × sapwood/heartwood	7.02	0.0025
Earlywood/latewood	16.25	0.0003
Species × earlywood/latewood	1.72	0.19
Sapwood/heartwood × earlywood/latewood	0.57	0.46

The focus of this study was analysis of inferred photosynthetic gas exchange over the last eight decades. Because of concern over potential influence of tree age being confounded with time (Craig 1954; Francey and Farquhar 1982; Yoder et al. 1994), we compared cellulose from trees of like age grown at different times. Wood from current saplings, formed between 1979 and 1991, was compared to wood from the inner rings of middle-aged trees, formed between 1941 and 1970, and mature trees, formed between 1910 and 1929. This experiment isolated the influences of conditions at the time the wood was formed and age of the tree in which the wood was formed.

Finally, trees of different age, but grown at the same time, i.e., current open-grown saplings versus recent wood from the tops of middle-aged and old trees, were compared to quantify the influence of age independent of the effects of source CO_2. Holocellulose was extracted from separate samples of earlywood and latewood as described above. Tree ages were estimated based on age of the oldest ring observed in the basal cross-section.

Inference of gas exchange variables

Plants using the C_3 photosynthetic pathway discriminate against ^{13}C relative to ^{12}C as they assimilate atmospheric CO_2. Discrimination varies with the ratio of c_i divided by c_a as follows (Farquhar et al. 1982):

$$\delta^{13}C_{\mathrm{plant}} = \delta^{13}C_{\mathrm{air}} - a - (b - a) * (c_i/c_a) \tag{2}$$

where $\delta^{13}C_{\mathrm{plant}}$ is the ratio of ^{13}C to ^{12}C in the plant tissue expressed relative to the PDB standard (‰), $\delta^{13}C_{\mathrm{air}}$ is the isotopic composition of atmospheric CO_2, *a* is fractionation by diffusion through stomata (4.4‰), and *b* is a fitted parameter determined mostly by the fractionation of the carboxylating enzymes (27‰) (Farquhar and Richards 1984). Because *b* in Eq. 2 was fitted with whole-tissue isotopic data rather than cellulose data, we modified the cellulose $\delta^{13}C$ by adding the mean offset between cellulose and whole sapwood using the value for each species and for earlywood or latewood, as appropriate (Table 2). Although this might seem to negate the value of extracting cellulose in the first place, it does not. The cellulose extraction removed all carbon deposited after the annual ring first appeared; thus we removed any contamination by younger starch in sapwood or extractives in heartwood. Correction to whole sapwood values by the species-specific offsets in Table 2 justified our use of the value 27‰ for *b* in Eq. 2. Most previous studies have inferred gas exchange parameters directly from cellulose isotopic data; we present the results of such analyses for comparison to the adjusted data.

Annual records of past $\delta^{13}C_{\mathrm{air}}$ and c_a were obtained from published data from Antarctic ice cores (Friedli et al. 1986) and direct measurements summarized by Keeling et al. (1989). The ice core data have been corroborated by the isotopic record in cellulose of C_4 plants (Marino and McElroy 1991). Where necessary the data were extrapolated. The ice core data were entered into Eq. 2 along with the tree ring isotope data ($\delta^{13}C_{\mathrm{plant}}$) and the equation was solved for c_i. Simple rearrangement of Eq. 1 demonstrates that the difference $c_a - c_i$ equals *A*/*g*, also called intrinsic water use efficiency.

The data were analyzed by analysis of variance, treating species and either year, tree age, or atmospheric $[CO_2]$ as independent variables. Dependent variables included c_i, c_i/c_a, and c_a–c_i. The

Table 2 Mean difference in stable carbon isotope composition ($\delta^{13}C$) of cellulose and whole wood by tissue type Standard errors in parentheses; *n* = 4 replicates per factorial combination

Species	Sapwood		Heartwood	
	Earlywood	Latewood	Earlywood	Latewood
Douglas-fir	1.75 (0.06)	2.00 (0.15)	1.66 (0.12)	2.02 (0.19)
Ponderosa pine	0.65 (0.49)	2.25 (0.16)	2.28 (0.48)	2.78 (0.27)
Western white pine	2.13 (0.24)	2.64 (0.31)	1.65 (0.29)	2.40 (0.22)

interactions were also tested for significance to determine whether species differed in their responses.

Results

Comparison of saplings grown at different times

We observed a significant decrease in isotopic composition of cellulose of saplings during the twentieth century (-0.029‰ year^{-1}; SE = 0.003‰ year^{-1}). After correcting for differences in the isotopic composition of source air and for the offset between cellulose and whole sapwood, the ratio c_i/c_a was calculated. The ratio increased from 0.569 (SE = 0.006) to 0.624 (0.009) to 0.634 (0.006) for sapling rings from the oldest, middle-aged, and youngest trees, respectively (Tables 3, 4). Similarly, c_i increased over the study period from 171 (SE = 2) to 195 (3) to 219 (2) ppm (Tables 3, 4). In contrast, the change in c_a–c_i was not statistically significant; c_a–c_i averaged 127 (1) ppm throughout the period (Tables 3, 4). In no instance were there statistical differences among species, nor were there statistically significant interactions (Table 3a). The c_i estimates are presented versus year in which the wood was formed in Fig. 1. For comparison, Fig. 1 also presents theoretical estimates of c_i if c_i/c_a, c_a–c_i, and c_i were held constant at the levels of the oldest annual rings. The grouping of the data about the constant c_a–c_i curve graphically illustrates the lack of significant variation in c_a–c_i.

Analysis of the gas-exchange variables inferred from the raw holocellulose data yielded generally similar results (Tables 3, 4). *F*-values were lower and the error sum of squares was greater in the analyses of c_i/c_a and c_i; however, the *F*-values were greater for the analysis of c_a–c_i. The mixture of higher and lower *F*-values following the correction leads us to suggest that neither approach is obviously better in terms of the statistical fit to the data. The only difference in the conclusions one would draw is that the change in c_a–c_i was found to be statistically significant ($F = 4.94$, $P = 0.028$) in the analysis of the uncorrected data. The slope, however, was only 0.065‰ year^{-1}, which would account for a 5.2 ppm shift over 80 years. Perhaps the most important consequence of the adjustment is that it shifts c_i estimates upward by 25–30 ppm (Table 3).

Analyses of variance using c_a to replace year as the independent variable yielded results (not presented) quantitatively similar to those described above. We present regression equations (Fig. 2a–c) relating

Table 3 Mean $\delta^{13}C$, c_a, Δ, and sample size *n*, of sapling cellulose deposited during different periods of the twentieth century. The gas exchange variables c_i/c_a, c_i, and c_a–c_i were inferred following adjustment of all isotopic data to values typical of whole sapwood and directly from raw cellulose data

	Year of cellulose deposition		
	1910–1929	1941–1970	1988–1991
$\delta^{13}C$ (‰)	−22.29 ± 0.13	−23.52 ± 0.21	−24.37 ± 0.14
c_a (ppm)	301.3 ± 0.4	312.3 ± 0.7	346.2 ± 0.4
Δ (‰)	17.68 ± 0.14	18.97 ± 0.22	19.23 ± 0.14
n	80	30	72
Corrected to whole sapwood			
c_i/c_a (dimensionless)	0.569 ± 0.006	0.624 ± 0.009	0.634 ± 0.006
c_i (ppm)	171 ± 2	195 ± 3	219 ± 2
c_a–c_i (ppm)	130 ± 2	118 ± 3	127 ± 2
Raw cellulose			
c_i/c_a (dimensionless)	0.491 ± 0.005	0.541 ± 0.006	0.546 ± 0.007
c_i (ppm)	148 ± 2	169 ± 2	189 ± 3
c_a–c_i (ppm)	153 ± 2	143 ± 3	157 ± 2

Table 4 ANOVA table for c_i/c_a, c_i, and c_a–c_i inferred from sapling cellulose $\delta^{13}C$ (both raw cellulose and cellulose corrected to whole sapwood) deposited throughout the twentieth century. Year of deposition was analyzed as a covariate for data not corrected to whole sapwood rather than grouping according to the columns used for data that *was* corrected to whole sapwood. Error *df* = 176

	df	$\delta^{13}C$		Δ		c_i/c_a		c_i		c_a–c_i	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Corrected to whole sapwood											
Species	2	0.36	0.70	0.29	0.75	0.29	0.75	0.12	0.89	0.18	0.84
Year	1	85.40	< 0.0001	39.97	< 0.0001	30.86	< 0.0001	227.54	< 0.0001	0.94	0.33
Species × year	2	0.34	0.72	0.24	0.79	0.24	0.78	0.12	0.89	0.15	0.86
Raw cellulose											
	df										
Species	2					0.28	0.76	0.09	0.91	0.23	0.79
Year	1					24.98	< 0.0001	163.62	< 0.0001	4.94	0.03
Species × year	2					0.24	0.78	0.07	0.93	0.20	0.82

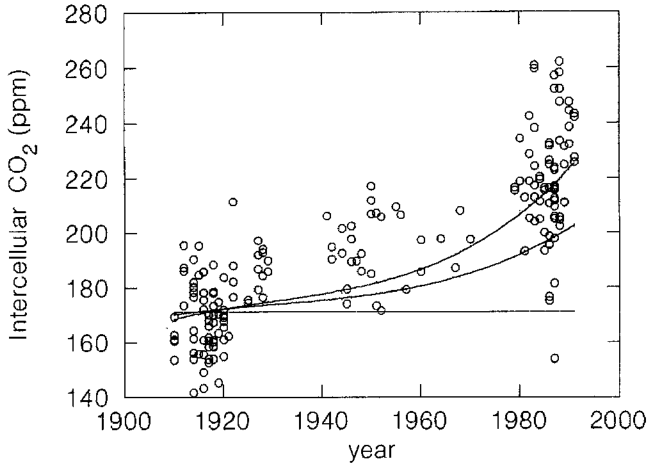

Fig. 1 Intercellular CO_2 concentrations (*open circles*) inferred from measured tree-ring isotopic data and published ice-core data for three species of conifer saplings in northern Idaho since 1910. Theoretical curves are also presented that show the expected variation in intercellular CO_2 if c_a–c_i were held constant (*top curve*), if c_i/c_a were held constant (*middle curve*), and if c_i were held constant at the mean values for the oldest saplings (*bottom line*)

ambient CO_2 concentrations to c_i/c_a and c_i. The relationship with c_a–c_i was not statistically significant. It follows that the slope of the regression estimating c_i from c_a is not significantly different from 1 (Fig. 2b).

Comparison of trees differing in age

Age-related differences in inferred gas-exchange characteristics were detected by comparing recently deposited wood from near the tops of mature trees, intermediate trees, and saplings (Table 6). Differences were observed in all three inferred gas-exchange variables with *P* values all less than 0.02. The ratio c_i/c_a decreased from 0.634 (0.007) to 0.577 (0.010) to 0.566 (0.006) for saplings, middle-aged trees, and old trees, respectively (Table 5). Intercellular [CO_2] decreased from 219 (2) to 196 (4) to 192 (2) ppm (Table 5). The [CO_2] differential increased from 127 (2) to 144 (4) to 148 (2) ppm (Table 5). There were once again no differences among species and there were no interactions between species and age (Table 6).

As a consequence of offsetting increases in c_i/c_a over time and decreases with age, changes in isotope composition over the lifetimes of the large trees have been inconsequential. In fact, analysis of variance of the inner rings near the base and the outer rings of the upper crown detected no significant difference in Δ ($n = 165$, $F = 0.83$, $P > F = 0.36$). Thus, it was only by analyzing the separate influences of environment and age that evidence of adjustment in photosynthetic gas-exchange parameters was detected.

Discussion

Three species of field-grown conifers have undergone detectable shifts in photosynthetic gas exchange during this century. The difference in [CO_2] across the stomata (c_a–c_i, or intrinsic water use efficiency) has remained constant despite a 60 ppm rise in ambient [CO_2]. Conservation of c_a–c_i was achieved by allowing c_i to drift upward at a rate equal to the rise in ambient [CO_2]; as a consequence, c_i/c_a also drifted upward.

The observed homeostasis of c_a–c_i would also be expressed as constant p_a–p_i, where p_a represents ambient CO_2 partial pressure and p_i represents intercellular CO_2 partial pressure, because all trees in this study were sampled from within a narrow altitudinal band. Körner et al. (1988, 1991) first reported the tendency for p_a–p_i to be held constant over altitudinal gradients in a global survey across multiple species; the tendency was particularly obvious among trees. Marshall and Zhang (1994) observed the same tendency across altitudinal gradients for the species in this study, among others. Maintenance of the partial pressure gradient was

Table 5 Mean $\delta^{13}C$, Δ, c_i/c_a, c_i, c_a–c_i, and sample size *n*, of recently deposited cellulose collected near the tops of the trees between 1988 and 1991

	Young	Intermediate	Old
$\delta^{13}C$ (‰)	−24.37 ± 0.15	−23.19 ± 0.23	−23.04 ± 0.14
Δ (‰)	19.23 ± 0.16	17.89 ± 0.25	17.62 ± 0.15
c_i/c_a (dimensionless)	0.634 ± 0.007	0.577 ± 0.010	0.566 ± 0.006
c_i (ppm)	219 ± 2	196 ± 4	192 ± 2
c_a–c_i (ppm)	127 ± 2	144 ± 4	148 ± 2
n	72	30	83

Table 6 ANOVA table for Δ, c_i/c_a, c_i and c_a–c_i, of cellulose deposited near the tops of trees of different age between 1988 and 1991. Error *df* = 179

	df	$\delta^{13}C$		Δ		c_i/c_a		c_i		c_a–c_i	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Species	2	0.38	0.68	0.37	0.69	0.36	0.70	0.50	0.61	0.37	0.69
Year	1	17.48	< 0.0001	13.22	0.0004	13.13	0.0004	28.46	< 0.0001	6.70	0.01
Species × year	2	0.38	0.68	0.38	0.69	0.37	0.69	0.50	0.61	0.37	0.69

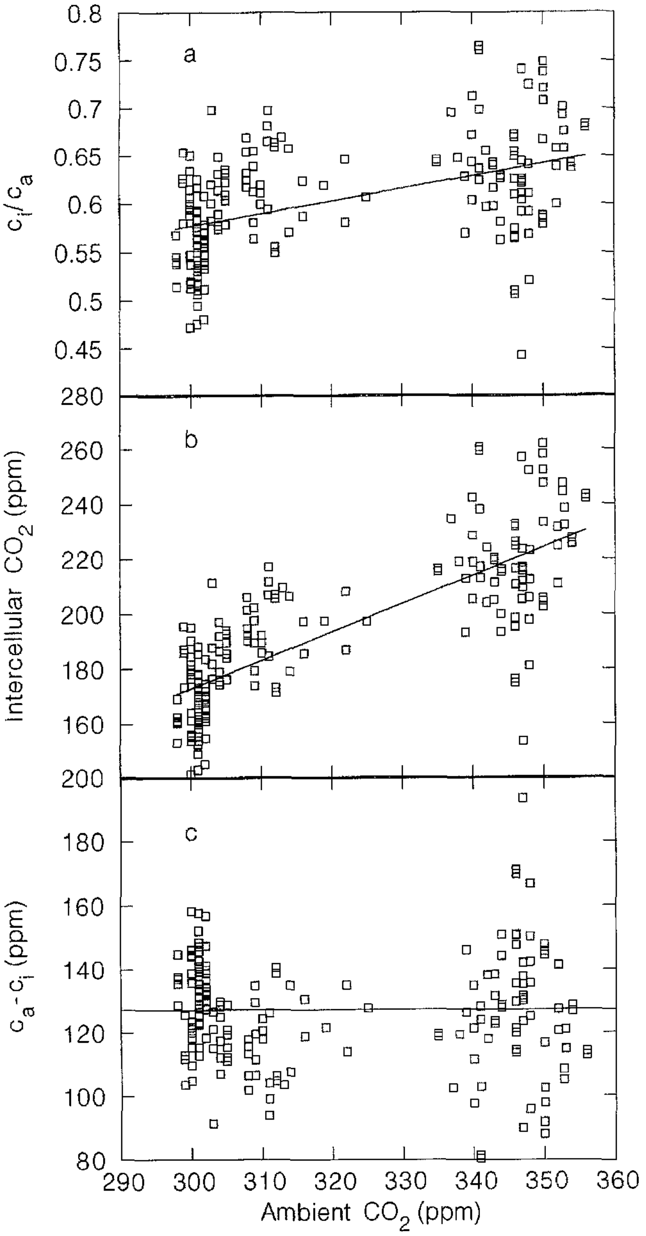

Fig. 2 Inferred c_i/c_a, c_i, c_a–c_i of three species of conifer saplings as a function of ambient CO_2 concentration of the bulk atmosphere. Regression equations: **a** c_i/c_a (dimensionless) = 0.184 + 0.00131 [ambient CO_2 concentration (ppm)], $n = 182$, $r^2 = 0.21$, $P < 0.0001$; **b** c_i (ppm) = −135.8 + 1.029 [ambient CO_2] concentration (ppm)], $n = 182$, $r^2 = 0.61$, $P < 0.0001$; **c** slope not significant for c_a–c_i and intercept = 127

associated with a progressive decrease in isotopic discrimination as p_a declined with altitude. Thus both altitudinal pressure gradients and the change in CO_2 mole fraction over time have resulted in similar homeostatic adjustment of gas exchange. That the response by the trees is so similar leads us to suggest that the homeostatic adjustment may be a general phenomenon.

Marshall and Zhang (1994) found homeostatic set points for p_a–p_i at 11.6, 11.9, and 11.8 Pa, for Douglas-fir, ponderosa pine, and western white pine, respectively. As in this study, they were unable to detect differences among these three species. Quantitatively comparing their data with those reported here requires that the [CO_2] differential, 127 ppm, be multiplied by barometric pressure (91 kPa) yielding 11.4 kPa. A similar calculation using the uncorrected holocellulose data yields 13.7 kPa. Based on the better agreement of the adjusted data with earlier work on whole leaves (Marshall and Zhang 1994) we suggest that the adjustment of the ring cellulose data by the offsets in Table 2 improves the accuracy of the inference of gas-exchange parameters.

Interestingly, however, when the c_a–c_i data were analyzed by group (old versus middle-aged versus young) significant differences were observed ($F = 5.90$, $P = 0.0033$); the sapling rings of intermediate age averaged 118 (SE = 3) ppm while the sapling rings of old trees and young trees had values of 130 (2) and 127 (2), respectively. We know less about the conditions under which the intermediate saplings grew than we do about the older and the younger trees. The old trees regenerated after a stand-replacing fire and the youngest trees were selected only if they occurred under open-grown conditions, i.e., where overstory trees had been cut to build a forest road. In contrast, the intermediate trees are more likely to have grown under partial shade, which would tend to reduce c_a–c_i (Francey and Farquhar 1982; Ehleringer et al. 1986).

Conifer tree rings have, with few exceptions, undergone an isotopic shift of −1.2 to −2.0‰ in the past century. For comparison, atmospheric $\delta^{13}C$ has shifted by about −1.1‰ since the turn of the century, and −1.5‰ over the past 400 years (Keeling et al. 1989; Marino and McElroy 1991). Particularly noteworthy in this respect are the data of Leavitt and Long (1989), summarized from 56 pinyon pine trees from before 1600 a.d. These data, which were corrected for climate variation based on ring width, show stable $\delta^{13}C$ for three centuries, until about 1900 a.d., after which $\delta^{13}C$ begins to shift by about −1.2‰. They further show that the shift corresponds with shifts in the isotopic composition of atmospheric CO_2. Thus, discrimination would not have changed. In fact, in a later paper Leavitt and Lara (1994) conclude that c_i/c_a has remained approximately constant in old Chilean *Fitzroya* trees. Similar negative shifts in tree ring $\delta^{13}C$ of about −2‰ have been reported for Scots pine (Freyer and Belacy 1983), bristlecone pine (Leavitt and Long 1992), and a large number of conifers between 1850 and the present (Stuiver and Braziunas 1987). Martin and Sutherland (1990) observed shifts of −1.4‰ for the first eight decades of the twentieth century in Douglas fir. A clear exception to the above pattern is among Tasmanian conifer species, which had not shifted in isotopic composition as of 1970 (Francey 1981). Francey and Farquhar (1982) noted that the Tasmanian results suggested that c_i had been held constant.

We assume that $\delta^{13}C$ of the air in the immediate vicinity of our sample trees during periods of rapid photosynthesis is the same as mean atmospheric $\delta^{13}C$ estimated from the ice core data. Because of ^{13}C discrimination during photosynthesis in C_3 plants, roots and decomposing soil organic matter respire CO_2 that is isotopically lighter than the atmosphere. Respired CO_2 has a strong influence on $\delta^{13}C$ close to the ground, but that influence decreases sharply with increasing height above the ground (Francey et al. 1985; Sternberg et al. 1989) and is less pronounced in temperate conifer canopies than in broadleaved or tropical forests (Broadmeadow and Griffiths 1993). Conifer canopies are well coupled to the bulk atmosphere because of their surface roughness and the high boundary layer conductance of their narrow leaves (Jarvis and McNaughton 1986). Moreover, the modern saplings were sampled at heights of at least 1.4 m and were chosen for sampling based on the absence of taller trees that might otherwise have interfered with fluxes of light or CO_2. Ponderosa pine is decidedly shade intolerant, and Douglas-fir and western white pine are intermediate in shade tolerance, so saplings of these species in established stands are usually found in openings that would be well coupled to the atmosphere.

Few researchers have worked with material from saplings, perhaps out of concern that the carbon isotope ratios so near the ground would be different from those in the canopy. In spite of this, we see a great advantage in working with sapling material, for it allows us to break the confounding between age and time. Because we compared rings laid down by trees in different times at the same stage of development, we were able to detect shifts in gas exchange over the course of this century.

A separate analysis provided a direct test of the "juvenile effect" on carbon isotope ratios corrected for variation in source CO_2 (Francey 1981). The trees in our study decreased in c_i/c_a from 0.63 to 0.57 as they progressed from saplings to middle-aged trees. In the absence of a change in source CO_2, such a decrease in c_i/c_a would induce an increase in $\delta^{13}C$ of about 1.4‰ (Fig. 2). Craig (1954) observed a shift of about 2‰ early in the life of *Sequoia* trees. Francey and Farquhar (1982) reported a sudden shift of about 2% associated with fire scars early in the life of Tasmanian trees. Freyer (1979) reported a shift or about 1.4‰ in *Pinus sylvestris*. Yoder et al. (1994) observed that 40-m tall trees were about 2‰ less negative than 2-m tall trees in an open ponderosa pine stand. Thus, both the direction and magnitude of our estimate of the juvenile effect agree with previous findings.

We have emphasized the analysis of the $\delta^{13}C$ data corrected to whole sapwood values prior to the inference of gas-exchange parameters. This correction was performed out of concern for the progressive enrichment of $\delta^{13}C$ values from whole tissue to cellulose and from leaves to stems. These issues are discussed in relation to the utility of paleodata by White et al. (1993). Fortunately, the adjustment had little influence on the conclusions – trends were similar even without the correction. The only difference between the analyses was that a slight increase in c_a–c_i (3% over 80 years) was detected in the uncorrected data. This difference is so small in comparison to the changes in c_i (33%) and c_i/c_a (13%) that we would stand by our conclusion that c_a–c_i is homeostatically maintained even if the raw cellulose data were demonstrated to be the more rigorous analysis.

Our interpretation has assumed that the observed gas exchange response is attributable to the global change in atmospheric [CO_2]. With careful selection of sample stands and trees, we were able to account for variation due to age, time, position in the tree, and species. Variation due to temporal changes in chemical composition, i.e., heartwood formation, was removed by cellulose extraction. In addition, annual cycles of water use efficiency were accounted for by separately analyzing samples of earlywood and latewood (Leavitt and Long 1986). We assume that air pollution has had little effect because the site is so isolated; the only nearby urban area is Spokane, Washington, a city of approximately 200,000 at a distance of 80 km. There is no evidence of a trend in the precipitation records, which have been maintained at Priest River over the entire period of interest (Earthinfo Inc 1992). It is not likely that observed tree ring response can be attributed to a change in nutrient status because analyses of fertilizer effects have previously shown no significant influence on water use efficiency of Douglas-fir (Mitchell and Hinckley 1993) or ponderosa pine (J. Marshall and R. Korol, unpublished data).

The traditional alternative to field studies is controlled greenhouse experiments that try to control confounding factors. Evidence from CO_2-enrichment experiments, which typically increase CO_2 partial pressures by 30–60 Pa, suggests that increasing partial pressure of CO_2 generally increases A/g of C_3 plants (Eamus and Jarvis 1989; Polley et al. 1993), sometimes dramatically (Idso and Kimball 1992). However, these increases in A/g are not always observed among conifers (Eamus and Jarvis 1989) and ponderosa pine has previously been reported to show no long-term gas-exchange response to elevated CO_2 (Grulke et al. 1993). On the other hand, other studies have found significant gas-exchange effects in both ponderosa pine (Surano et al. 1986; Callaway et al. 1994) and Douglas-fir (Hollinger 1987). Our results from forest-grown trees uncovered no increase in c_a–c_i over the smaller increase in CO_2 partial pressures (6 Pa) in the ambient atmosphere during this century.

The utility of published CO_2 enrichment experiments is to some extent compromised by the artificiality of the exposure conditions and the near-total reliance on seedlings. Tree rings provide a valuable adjunct to chamber studies of CO_2 response because they yield data describing growth under natural conditions. Trees growing in the field occur in systems with all feedbacks in place; if CO_2 enrichment influences insect feeding, mycorrhizal colonization, or nutrient mineralization, these influences will be difficult to account for in controlled environment studies. Moreover tree rings can be obtained from large trees as well as seedlings, and thus do not depend on the assumption that large trees and seedlings behave similarly.

During the 20th century the $[CO_2]$ of the ambient atmosphere (c_a) has increased 60 ppm. Our objective was to examine the effect on gas exchange contemporaneous with the increase in atmospheric $[CO_2]$. Our results from forest-grown trees uncovered significant changes in c_i and c_i/c_a but no change in c_a–c_i. The observed maintenance of constant c_a–c_i has required an upward shift in c_i equal to the increase in c_a. These results provide evidence that these field-grown forest trees have undergone a homeostatic physiological response to maintain constant c_a–c_i despite the rise in atmospheric CO_2 during the twentieth century.

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