

Is there a stable isotope evidence for the CO₂ fertilization effect?

R V KRISHNAMURTHY and M MACHAVARAM

*Department of Geology, Western Michigan University, Kalamazoo, MI 49008, USA
email: r.v.krishnamurthy@wmich.edu*

It has been suggested that part of the so-called "missing sink" of carbon dioxide introduced into the atmosphere by anthropogenic activities, that is the imbalance between estimated anthropogenic carbon dioxide emissions and oceanic uptake, may be stored in the vegetation in midlatitudes. Precise mechanisms of abstraction of additional carbon dioxide by vegetation, also known as the "fertilization effect", are poorly understood. Stable carbon and hydrogen isotope ratios of cellulose extracted from annual growth rings (covering the time period 1980–1993) in an oak tree from Kalamazoo, SW Michigan provide a basis to investigate at a physiological level how the fertilization effect may operate. The carbon isotope ratios show that the intercellular concentration of carbon dioxide increased due to an increase in stomatal opening. Although increased intercellular concentration of carbon dioxide translated to increased Water Use Efficiency and assimilation rates, it also resulted in increased transpiration rate as shown by higher D/H of the fixed carbon. The two-fold significance of the isotope data are: first, they provide the first field evidence based on isotope studies for excess CO₂ induced biomass production and second, they suggest that this mechanism is likely to operate only in limited environments. Vegetation in regions where moisture availability is not restricted so that there can be a gain in water use efficiency despite increased leaf evaporation are best suited to sequester excess carbon from the atmosphere.

1. Introduction

Recent estimates show that between 1980–1989, the total emission of CO₂ was equivalent to about 6.3×10^{16} g of C (Dixon *et al* 1994). Of these, 2.88×10^{16} g of carbon remains in the atmosphere and the oceans are estimated to have absorbed about 1.8×10^{16} g of C. The amount of carbon that is unaccounted for is thus estimated to be 1.62×10^{16} g. It has been suggested that low and mid-latitude terrestrial ecosystems may be significant repositories of this missing carbon, the so-called "missing sink" of atmospheric CO₂. The process in which more dry matter is produced by plants via increased photosynthetic activity is also sometimes called the "fertilization effect". Consequently, fertilization effect can in principle abstract large amounts of CO₂ and sequester as vegetation. Fertilization effect in vegetation in these ecosystems should be accompanied by physiological

responses a knowledge of which will enable us to identify regions of the world where this might be taking place. At present growth chamber experiments are the main focus in studies related to this important process. Here recent trends in the carbon and hydrogen isotope ratio (expressed as δ values) of annual growth rings from an oak tree in Kalamazoo (42.10 N, 85.30 W), southwestern Michigan has been used to demonstrate how the fertilization effect might operate in the natural environment.

2. Results and discussion

Samples representing individual years from an oak tree cut in 1993 were analyzed for $\delta^{13}\text{C}$ and δD after extracting cellulose from the whole wood. A total number of 47 individual rings (1947–1993) were analyzed. For all the samples both the $\delta^{13}\text{C}$ and δD

Keywords. Carbon isotopes; intercellular carbon dioxide; fertilization effect; hydrogen isotopes.

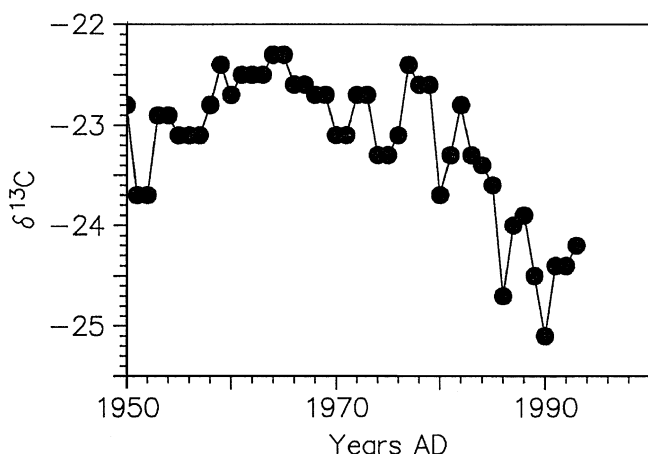


Figure 1. Carbon isotope ratio of cellulose from an oak tree from Kalamazoo, SW Michigan as a function of time.

were determined. The $\delta^{13}\text{C}$ was obtained by combustion of cellulose extracted from the raw wood while the δD values were determined on the cellulose using an equilibration technique that involved heating the samples at 180°C for 15 hrs (Krishnamurthy and Machavaram 1998). The $\delta^{13}\text{C}$ record for the period 1950–1993 is shown in figure 1. The isotope values ($\delta^{13}\text{C}$ and δD) did not correlate with any of the climatic variables such as annual temperature or precipitation or growing season temperature or precipitation recorded in Kalamazoo (table 1). This is particularly striking in the case of δD which might be expected to record climate information (Yapp and Epstein 1982; Krishnamurthy and Epstein 1985; Ramesh *et al* 1986; Epstein and Krishnamurthy 1990).

The interpretation of $\delta^{13}\text{C}$ of plant carbon has been made somewhat simpler by a widely accepted carbon isotope fractionation model (Farquhar *et al* 1989).

The fractionation model is given by:

$$\delta^{13}\text{C}_p = \delta^{13}\text{C}_{\text{air}} - a - (b - a) \frac{C_i}{C_a} \quad (1)$$

where,

$\delta^{13}\text{C}_p$ = the isotope ratio of the fixed carbon (photosynthate)

$\delta^{13}\text{C}_{\text{air}}$ = the isotope ratio of the source i.e. atmospheric CO_2

a = the fractionation due to diffusion of CO_2 into the leaf (4.4)

b = fractionation due to fixation of CO_2 via enzyme action (~ 27 for C_3 plants)

C_i & C_a = Concentration of CO_2 in the leaf intercellular space and in the atmosphere.

The above model applies to fractionation at the leaf and ignores possible isotope effects, apparently a systematic one, between leaf and tissue matter (Leavitt and Long 1982). This would not affect the applicability of the model in the present context as long as this effect is systematic. The C_i/C_a ratio is a very useful index of stomatal control on photosynthesis and is also tightly connected to WUE, Water Use Efficiency (Farquhar and Richards 1984, Farquhar *et al* 1988; Ehleringer 1988). If A is the instantaneous rate of carbon assimilation and E the transpiration rate, A/E defines the water-use efficiency (WUE) A with:

$$W = \frac{A}{E} = \frac{C_a - C_i}{1.6\nu} \quad (2)$$

where C_a and C_i are concentrations of CO_2 in the atmosphere and intercellular space respectively; ν = the difference in vapor pressures in the intercellular space and the atmosphere, 1.6 being the ratio of diffusivities of water vapor and CO_2 in air. WUE being a function of the gross assimilation rate, $C_a - C_i$ should have a direct bearing on the assimilation rate. In

Table 1. Isotopic and climatic values from Kalamazoo, SW Michigan, for the period 1980–1993. Isotope values are based on the analysis of an oak tree. Also given are the estimated C_i/C_a and $C_a - C_i$ values obtained using the carbon isotope fractionation model.

| Year | Mean (Apr-Oct) CO_2 (ppm) | Mean (Apr-Oct) $\delta^{13}\text{C}$ atm | $\delta^{13}\text{C}$ of cellulose | δD of cell | Mean temp. (Apr-Oct) in $^\circ\text{C}$ | Mean precip. (Apr-Oct) in cm | Mean annual precipitation in cm | Ring width mm | C_i/C_a | $C_a - C_i$ ppm based on C_i/C_a 0.524 |
|------|------------------------------------|--|------------------------------------|--------------------------|--|------------------------------|---------------------------------|---------------|-----------|--|
| 1980 | 338.75 | -7.58 | -23.7 | -66 | 19.84 | 69.28 | 114.25 | 10.3 | 0.52 | 161.3 |
| 1981 | 339.85 | -7.64 | -23.3 | -65 | 19.53 | 48.35 | 93.1 | 5.8 | 0.50 | 161.8 |
| 1982 | 341.08 | -7.58 | -22.8 | -77 | 19.39 | 57.85 | 123 | 8.4 | 0.48 | 162.5 |
| 1983 | 343 | -7.68 | -23.3 | -66 | 20.19 | 50.5 | 110.1 | 11 | 0.50 | 163.7 |
| 1984 | 344.45 | -7.75 | -23.4 | -70 | 19.21 | 38 | 93.4 | 9.4 | 0.50 | 164 |
| 1985 | 345.92 | -7.66 | -23.6 | -71 | 19.31 | 45.25 | 124.48 | 9.6 | 0.51 | 164.7 |
| 1986 | 347.28 | -7.63 | -24.7 | -73 | 19.64 | 81.4 | 130.4 | 11.7 | 0.56 | 165.3 |
| 1987 | 349.04 | -7.71 | -24 | -65 | 21.14 | 51.63 | 98.33 | 10.4 | 0.53 | 166.4 |
| 1988 | 351.53 | -7.80 | -23.9 | -60 | 21.21 | 43.35 | 112.13 | 8.5 | 0.52 | 167.3 |
| 1989 | 352.88 | -7.81 | -24.5 | -54 | 19.02 | 71.75 | 117.2 | 10.2 | 0.54 | 168 |
| 1990 | 354.01 | -7.80 | -25.1 | -55 | 18.64 | 56.76 | 137.63 | 7 | 0.54 | 168.5 |
| 1991 | 355.68 | -7.80 | -24.4 | -55 | 20.49 | 46.1 | 127.75 | 6.9 | 0.54 | 169.3 |
| 1992 | 356.54 | -7.83 | -24.4 | -65 | 17.72 | 48.18 | 108.08 | 7.1 | 0.54 | 169.7 |
| 1993 | 357.11 | -7.77 | -24.2 | -58 | 19.04 | 67.33 | 122.88 | 9.5 | 0.53 | 170 |

general, photosynthesis is an optimization between stomatal opening and transpiration. Efficient WUE requires minimal stomatal opening which in turn results in lower C_i and lower transpiration. This might be expected where moisture availability is a limiting factor. Since during the past century there has been a significant increase in atmospheric CO_2 concentration, it would be interesting to investigate how C_i/C_a , WUE and photosynthesis have been influenced in trees. This can provide insight into the response of terrestrial vegetation to elevated levels of CO_2 and address the fertilizer effect (LaMarche *et al* 1984).

In the next step involving the exploitation of the isotope fractionation model, the $\delta^{13}\text{C}$ of atmospheric CO_2 as measured in air ($\delta^{13}\text{C}_{\text{air}}$) is input into the photosynthesis model. Such direct measurements are available since 1978 for the Northern Hemisphere. The time period 1980–1993 was chosen in view of the fact that monthly measurements of $\delta^{13}\text{C}_{\text{air}}$ are available on a continuous basis for this time interval (Keeling *et al* 1989). In the application of the fractionation model, it is further assumed that the photosynthesis in this as well as in vegetation in general in these latitudes is significant only during the growing season (typically April–October for higher plants). Therefore, the $\delta^{13}\text{C}_{\text{air}}$ for these months only need be considered. The available values, kindly provided by Dr Keeling's group, are shown in table 1. Since the sampled tree is not from any forested area it is safe to assume that the tree processed free atmospheric CO_2 . This can also be verified by considering the significant correlation between the tree isotope record and the Northern Hemisphere growing season record of atmospheric CO_2 (figure 2). Such a good correlation would not be expected if the tree growth was controlled by local microenvironment. Also, since the isotope data of only the past 13 years are being used, interference from possible juvenile effects are absent. In these calculations the values of a , the diffusive fractionation factor and b , the enzyme related fractionation factors are taken as 4.4 and 27 respectively. In the final C_3 fixation, a value of 27 is preferred for b rather than 29 (Roeske and O'Leary 1984). This latter value was determined for Rubisco carboxylation with dissolved carbon dioxide as the source of carbon.

Using the $\delta^{13}\text{C}_{\text{air}}$ and the carbon isotope fractionation model a continuous C_i/C_a record for the time period 1980–1993 can be generated, as shown in table 1. It should be noted that C_i and C_a here represent the values for the growing season i.e. April–October. The interesting observation that emerges from this C_i/C_a record is that this parameter has remained fairly constant at 0.524 ± 0.025 over the past 13 years. This is not surprising since the strategy by plants would be to maintain a constant C_i/C_a ratio and thus $C_a - C_i$ to maintain optimum photosynthetic efficiency. Evidence for the tendency by plants to maintain a constant C_i/C_a over an even longer time scale has been shown

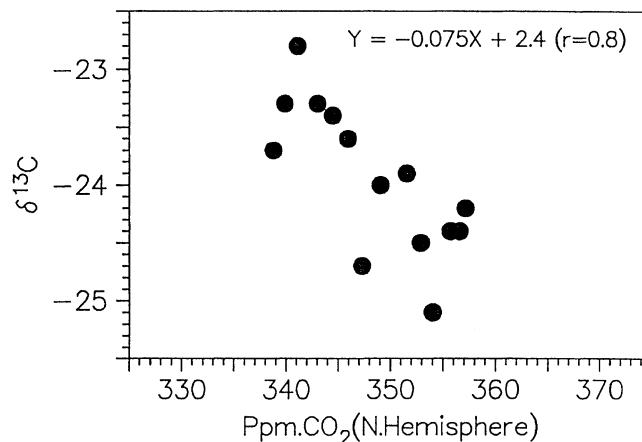


Figure 2. Relationship between the carbon isotope ratio of cellulose in oak and mean growing season (April–October) concentration of atmospheric carbon dioxide in the Northern Hemisphere.

(Krishnamurthy 1996). Since C_a , the concentration of external CO_2 has increased between the period 1980–1993, the constant C_i/C_a ratio would require that C_i and correspondingly $C_a - C_i$ also increased during this period. Table 1 shows the estimated change in $C_a - C_i$ during the past 13 years, calculated using the constant C_i/C_a of 0.524 and Keeling *et al* data of C_a . This data, also shown in figure 3, shows that $C_a - C_i$ has changed by about 5.4% during the past 13 years. Van de Water *et al* (1994) reported that $C_a - C_i$ increased by 13.6% between Glacial and Holocene periods, based on their study of limber pine needles (Van de Water *et al* 1994). It must be pointed out that the stomatal response to changes in external CO_2 and hence the effect on C_i/C_a and WUE is variable.

The increase in $C_a - C_i$, resulting from the tree's tendency to maintain a constant C_i/C_a , can be interpreted to be due to greater stomatal opening in response to the availability of higher amounts of CO_2 . Recent studies indicate that plants growing over a carbon dioxide range of ~ 200 – 350 ppm tend to conserve C_i/C_a ratios (Polley *et al* 1993; Feng and

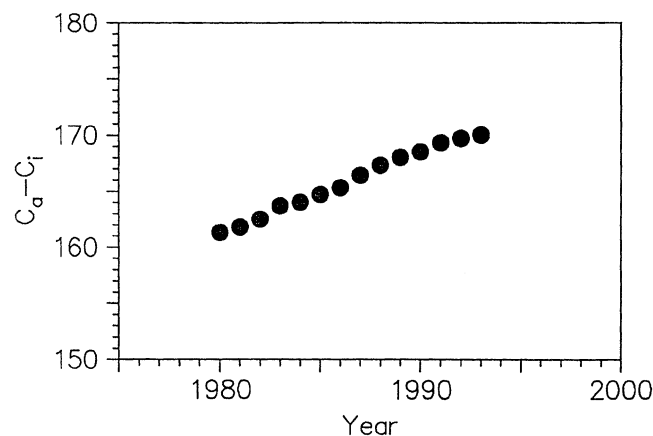


Figure 3. Estimated rate of increase in $C_a - C_i$ in the oak tree during the past fourteen (1980–1993) years.

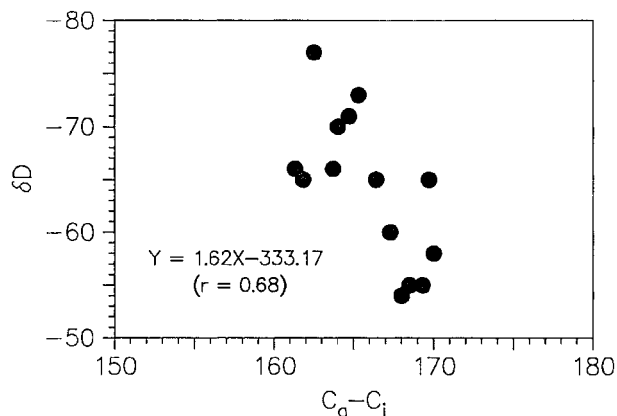


Figure 4. Relationship between δD and $C_a - C_i$.

Epstein 1995). If so, greater stomatal opening should result in greater water loss via evaporation at the leaf. This should result in the δD of the corresponding samples becoming higher as transpiration causes the water molecule with the lighter isotope i.e. hydrogen to escape preferentially. That this indeed is the case is deduced from figure 4 which shows the δD record for the same samples plotted against $C_a - C_i$ for the time period 1980–1993. In turn this should have an impact on the WUE since the latter is related to this parameter. The important observation is WUE and thus the assimilation rate appears to have increased despite a contradiction. The contradiction is that a greater stomatal opening should inhibit WUE since it means greater water loss. The justification for this may lie in the fact that the sample site is not a moisture stressed area where the plants would tend to preserve water by reducing stomatal opening, especially when greater amounts of CO_2 are available. Therefore it can be argued that in the present case, the sample site is not restricted in moisture availability which might favor an increase in intercellular CO_2 concentration at the rate of increased transpiration. This interpretation would suggest that a response by terrestrial ecosystems to increasing atmospheric CO_2 via increased assimilation rates i.e. CO_2 fertilization effect might best operate in regions where moisture stress is not an important factor.

Another interesting observation that resulted from this study is that the ringwidth did not show any significant correlation ($r=0.34$) with $C_a - C_i$. It is not clear if a greater assimilation rate *per se* should be reflected in ringwidth increase or that the increased biomass production can be in terms of plant fractions (e.g. leaf, foliage) that need not necessarily be converted into storable carbon (Condon *et al* 1987).

Acknowledgements

Supported by NSF grant ATM921369 and EAR9632034. We thank Dr Keeling's group for providing us with atmospheric data. We thank Dr R Ramesh for constructive review of the paper.

References

- Condon A G, Richards R A and Farquhar G D 1987 Carbon isotope discrimination is positively correlated with grain yield and dry matter production in field-grown wheat; *Crop. Sci.* **27** 996–1001
- Dixon R K, Brown S, Houghton R A, Solomon A M, Trexler M C and Wisniewski J 1994 Carbon pools and flux of global forest ecosystems; *Science* **263** 186–190
- Ehleringer J R 1988 Carbon isotope ratios and physiological processes in arid land plants; In: *Stable isotopes in Ecological Research* (eds) P W Rundel, J R Ehleringer and K A Nagy Ecological Studies 68, (Amsterdam: Springer-Verlag) pp 40–52
- Epstein S and Krishnamurthy R V 1990 Environmental information from the isotopic record in trees; *Phil. Trans. Roy. Soc. London* **A330** 427–439
- Farquhar G D and Richards R A 1984 Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes; *Aust. J. Plant Physiol.* **11** 539–552
- Farquhar G D, Ehleringer J R and Hubick K T 1989 Carbon isotope discrimination and photosynthesis; *Ann. Rev. Plant. Physiol. Plant Mol. Biol.* **40** 503–537
- Farquhar G D, Hubick K T, Condon A G and Richards R A 1988 Carbon isotope fractionation and plant water-use efficiency; In: *Stable isotopes in ecological research* (eds) P W Rundel, J R Ehleringer and K A Nagy, Ecological Studies 68, (Amsterdam: Springer-Verlag) pp 21–40
- Feng X and Epstein S 1995 Carbon isotopes of trees from arid environments and implications for reconstructing atmospheric CO_2 concentration; *Geochim. Cosmochim. Acta* **59** 2599–2608
- Keeling C D *et al* 1989 A three dimensional model of atmospheric CO_2 transport based on observed winds: 1. Analysis of observational data; *Am. Geophys. Monogr.*, No. 55 (ed) D H Peterson 165–235 (*Am. Geophys. Union*, Washington, DC)
- Krishnamurthy R V and Epstein S 1985 Tree Ring D/H Ratio From Kenya, East Africa and its paleoclimatic significance; *Nature* **317** 160–162
- Krishnamurthy R V 1996 Implications of a 400 year $^{13}C/^{12}C$ tree ring chronology; *Geophys. Res. Lett.* **23** 371–374
- Krishnamurthy R V and Machavaram M 1998 Hydrogen isotope exchange in thermally stressed cellulose; *Chem. Geol.* **152** 85–96
- LaMarche C V Jr, Graybill D A, Fritts C H and Rose M R 1984 Increasing atmospheric carbon dioxide: Tree ring evidence for growth enhancement in natural vegetation; *Science* **225** 1019–1021
- Leavitt S W and Long A 1982 Evidence for $^{13}C/^{12}C$ fractionation between tree leaves and wood; *Nature* **298** 742–744
- Polley H W, Johnson H B, Marino B D and Mayeux H S 1993 Increase in C_3 plant water-use efficiency and biomass over Glacial to present CO_2 concentrations; *Nature* **361** 61–63
- Ramesh R, Bhattacharya S K and Gopalan K 1986 Climatic correlations in the stable isotope records of silver fir (*Abies pindrow*) trees from Kashmir, India; *Earth Planet. Sci. Lett.* **79** 66–74
- Roeske C A and O'Leary M H 1984 Carbon isotope effects on the enzyme-catalyzed carboxylation of ribulosebiphosphate; *Biochemistry* **23** 6275–6284
- Van de Water P K, Leavitt S W and Betancourt J L 1994 Trends in stomatal density and $^{13}C/^{12}C$ ratios of pinus flexilis needles during last glacial-interglacial cycle; *Science* **264** 239–243
- Yapp C J and Epstein S 1982 Climatic significance of hydrogen isotope ratios in tree cellulose; *Nature* **297** 636–639