

Limitations to carbon assimilation: gas exchange and carbon isotope ratios in plants

Instantaneous and Long-term estimates of limitations to carbon assimilation are necessary to obtain if we want to understand what limits productivity, yield, distribution, etc. in plants.

I. *Limitation to photosynthesis in C₃ plants*

- A. gas-exchange can be used to directly estimate the instantaneous limitations to photosynthesis: from an analysis of the relationship between photosynthesis (A) and the internal CO₂ concentration of the leaf (c_i), the so called "A-c_i curve" can be constructed (Fig.)
- B. the magnitude of a diffusional across a stomata-air gradient varies from 50-280 μL L⁻¹ CO₂ depending on species and environmental conditions (Fig.)
- C. stomatal limitation to photosynthesis (I_s) can be estimated from the A-c_i curve:

$$I_s = 100 \cdot \left[\frac{A_{350} - A_{c_i}}{A_{350}} \right] \quad \text{in percent (\%)}$$

stomatal limitation can represent 10-60% of the total limitation to carbon fixation; the calculation method shown above has very little regard for the role of biochemical controls

- D. "Biochemical control theory" has also been used to estimate limitations to A. The model assumes that the flux of CO₂ in a plant occurs through a system composed of stomata and biochemical capacities. The biochemical capacities comprise all of the components of carbon metabolism after CO₂ has entered through stomata into the intercellular air spaces (from Ball et al. 1987). The control coefficient (Ω) is an approximation of the total limitation to photosynthesis by stomata and is expressed as:

$$\Omega = \{1 - ((c_s - c_i) \cdot g^* - g_c - 0.5E) / A^*\} - 1$$

where c_s = CO₂ concentration at the leaf surface (mol mol⁻¹); c_i = intercellular CO₂ concentration (mol mol⁻¹); g_c = stomatal conductance to CO₂ (mol m⁻² s⁻¹); E = transpiration (mol m⁻² s⁻¹); g* = slope of g-c_i curve; A* = slope of A-c_i curve. Polynomial regressions are fitted to the data and the slopes of the A-c_i and g-c_i curves are used in the calculation of C_c.

Additionally, "curve-fitting procedures have also be very powerful for determining stomatal and biochemical influences on photosynthesis, especially under stress - (see Ball et al. 1987 and Flanagan and Jefferies 1989; Figs.). Recent evidence from *in situ* chlorophyll fluorescence and RuBisCo assays shows that these estimations are too simple - why? (E & F below).

- E. early studies incorrectly associated all of the reduction in photosynthetic rate under water stress to stomatal closure. That is, they assumed no biochemical changes occurred which influenced photosynthetic capacity (Fig. - salinity experiments)
- F. non-uniform ("patchy") stomatal closure can account for large reductions in A (Fig.) and become very important in how they influence the calculation of c_i - important issue!

- G. instantaneous measures may or may not really inform us about seasonal or long-term limitations to carbon assimilation
- H. carbon isotope composition can be used to directly estimate stomatal limitation in C₃ plants, especially in the long-term (see below)

II. Carbon isotope ratio is ratio of two stable isotopes of carbon

¹²C and ¹³C (¹¹C and ¹⁴C are radioactive and unstable)

- A. ¹³C is about 1.1 % of total carbon; ¹²C is about 98.9 % of total carbon
- B. amount of ¹³C/¹²C is measured as a ratio on a mass spectrometer (Fig.)

$$R = {}^{13}\text{C}/{}^{12}\text{C}$$

however, precisely measuring an absolute ratio (R) is difficult, but measuring a difference is easier; so we measure isotope composition of a sample relative to a standard [i.e., measure the difference in signal between the standard and the sample values]

the standard for carbon isotopes is the fossil *Belemnita americana* from the Pee Dee Formation in South Carolina [chosen by Harold Urey at University of Chicago many years ago] = PDB

- C. carbon isotope ratios ($\delta^{13}\text{C}$) are expressed in "per mil" notation (‰ ; parts per thousand)

$$\delta^{13}\text{C} = \left[\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] \cdot 1000 \text{‰}$$

biological values vary from about -5 ‰ to about -35 ‰

(R_{sample} is always depleted in ¹³C relative to the standard)

- C. One of the first observations in the late 1960's was that there were two classes of carbon isotope ratios in plants (Fig.)

$\delta^{13}\text{C}$ values of -11 to -15 ‰ = C₄ photosynthetic pathway plants

$\delta^{13}\text{C}$ values of -21 to -35 ‰ = C₃ photosynthetic pathway plants

III. Variation in carbon isotope ratios of plant materials can be used in ecological analyses

- A. In most grasslands (Kenya in Africa, Great Plains in North America, etc.), there are gradients in the %C₃ and %C₄ composition within a grassland.
 1. Kenya grassland is C₄ dominated at low elevations (warmer) and C₃ dominated at higher elevations (cooler) (Fig.)
- D. North American grasslands are C₄ dominated in the south (Texas), but C₃ dominated in the north (Manitoba).

IV. Carbon isotope ratios of animals

- A. "You are what you eat" (more or less; $\pm 0.5\%$) - carbon isotope ratios of animals are quite similar to the carbon isotope ratios of the plants they eat
1. carbon isotope ratio can be used to determine food selectivity by animals
 - a. In Kenya, different animals prefer different species (Table)
 - Wildebeest prefers C₄
 - Thompson's Gazelle prefers C₄
 - Grant's Gazelle prefers C₃
 - b. In Georgia old field, all combinations of food preferences are found (Table)
- B. Carbon isotope ratios can be used to reconstruct an animal's diet (using historical markers, anything that is laid down permanently; hair, fingernails, bone, shell, baleen) - or a plant's growth history (tree rings) - (Figs.)

V. Why carbon isotopic composition varies in plant materials

- A. carbon isotope composition (ratios) of plant tissues will depend upon (Fig.)
- atmospheric source ($\delta^{13}\text{C}_{\text{air}}$)
 - amount of discrimination that occurs enzymatically
 - temperature dependence of fractionation
 - diffusion rate differences into leaf and to carboxylation site
- B*. the final isotopic ratio in the tissue will be a function of the c_i/c_a ratio (Fig.) and can be expressed as either a **negative value** (actual carbon isotopic composition relative to the standard; the tissue value relative to air is always isotopically lighter or has a more negative $\delta^{13}\text{C}$ value) OR a **positive value** (reflecting the magnitude the plant discriminates one isotopic species over the other; more discrimination against the heavier carbon isotopes, ^{13}C , leads to more positive Δ values) - see formulas below
- C. carbon isotope ratio of CO_2 in the atmosphere ($\delta^{13}\text{C}_{\text{air}}$) is -7.8 to -8.1% (more - each year)
- D. carbon isotope ratio of C₃ plants varies from about -18% to about -35% (Figs.)
- depends on diffusion difference, $^{13}\text{CO}_2$ is slower than $^{12}\text{CO}_2$ ($a = 4.4\%$)
 - depends on RuBP carboxylase discrimination ($b = 27$ to 30%)
 - depends on intercellular CO_2 levels (c_i), or better the ratio of c_i to c_a
- $$\delta^{13}\text{C} = \delta^{13}\text{C}_{\text{air}} - a - (b - a) \cdot c_i/c_a$$
- E. carbon isotope values for C₃ plants have recently been expressed as a function of "discrimination" (Δ) by the leaf itself. This is a more meaningful expression because it measures the isotopic composition relative to the source of carbon plants use [atmospheric CO_2] rather than to an arbitrary standard PDB. Carbon isotope discrimination values are expressed as positive values, where:

$$\Delta = a + [(b - a) \cdot c_i / c_a]$$

- F. carbon isotope ratios of **C₄ plants** depends on "leakage rates" from apoplastic regions of bundle sheath cells (and hence refixation rates) and the fact that carbon is fixed twice [first as bicarbonate (not CO₂) by PEP carboxylase] and by RuBisco (Figs.) so that the Δ for C₄ species can be expressed as:

$$\Delta_{C4} = a + (b_4 + b_3 [\emptyset - a]) \cdot c_i / c_a$$

where b_3 = RuBP carboxylase discrimination (27 to 30 ‰) and b_4 = PEP carboxylase discrimination against bicarbonate (-5 ‰) and \emptyset is the leakage rate of CO₂ out of the bundle sheath cells (0.1 to 0.6‰)

C₄ leaf carbon isotope ratio varies from about -10 to -15 ‰ (or 2 to 7 ‰ as Δ)

PEP carboxylase does not discriminate against ¹³C to as large an extent as RuBisco, and so $\delta^{13}\text{C}$ values of C₄ plants are close to the sum of air (-8‰) and diffusion (-4.4‰) components

- G. carbon isotope ratios in CAM plants depends on whether the plants are obligate CAM or facultative CAM plants:

$\delta^{13}\text{C}$ ratio of CAM plants vary from about -12 to -22 ‰ (or 4 to 15 ‰ as Δ)

VI. Carbon isotope ratio in C₃ plants has been used to estimate water use efficiency

= the ratio of carbon gained via photosynthesis to water loss via transpiration (A/E)

photosynthesis $A = (c_a - c_i) \cdot g / 1.6$

transpiration $E = (e_i - e_o) \cdot g$

water-use efficiency $A/E = (c_a - c_i) / [1.6 \cdot (e_i - e_o)]$

ASSUMPTIONS: if (1) c_a is constant; (2) within a habitat ($e_i - e_o$) is constant; and (3) the proportion of carbon lost via R_d is the same, then the carbon isotope ratio is going to be proportional to c_i or to A/E - REMEMBER THAT Δ IS INVERSELY PROPORTIONAL TO A/E (Figs.) This can be used to understand how environmental stresses affect integrated water-use efficiency (Δ). NOTE: it is important to realize that the three assumptions stated above can rarely be met and as such, Δ is considered a *absolute* index of c_i / c_a and a *relative* index of A/E (see Dawson and Ehleringer, 1993).

VII. Carbon isotope ratios increase in response to drought stress

- Under water stress, stomata close (increasing the stomatal limitation, decreasing c_i , and therefore increasing water use efficiency (Fig.))
- Across sites in the desert, there is variation in the carbon isotope ratio of a single species, reflecting water status in that microhabitat (Fig.)
- Longer-lived species tend to have higher (less negative) carbon isotope ratios than do short-lived perennials (Table)

- D. Even trees within riparian zones can differ in their carbon isotope ratios reflecting aspects of the water-use efficiency and thus position along a soil moisture gradient (Table - *Acer negundo*)
- E. In understory plants of forest communities where decomposition can influence the isotopic composition of the air, "source" effects" can be seen - (Figs.)

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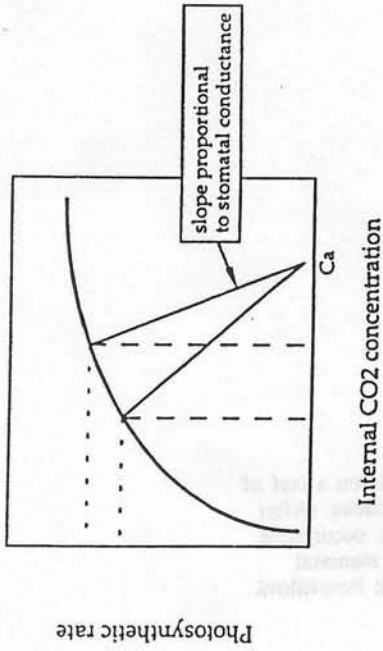
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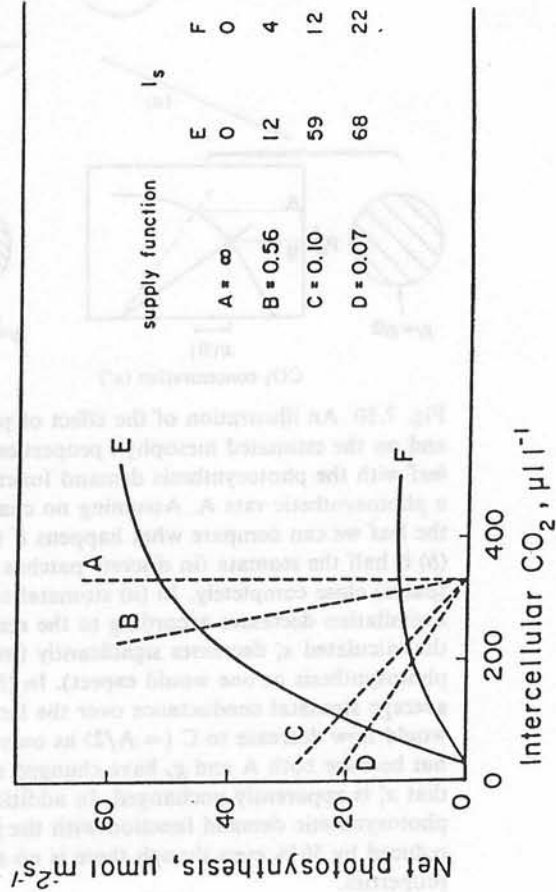
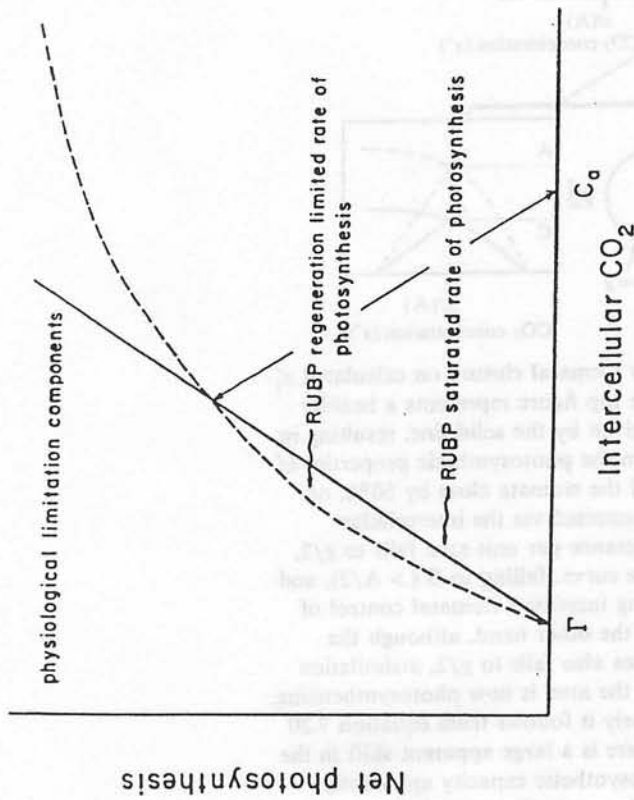
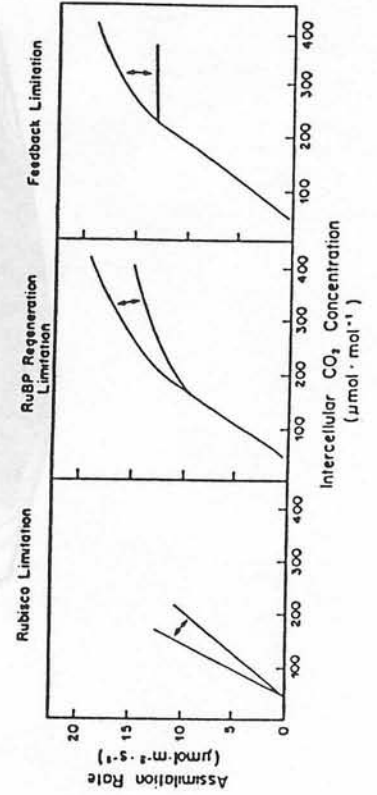
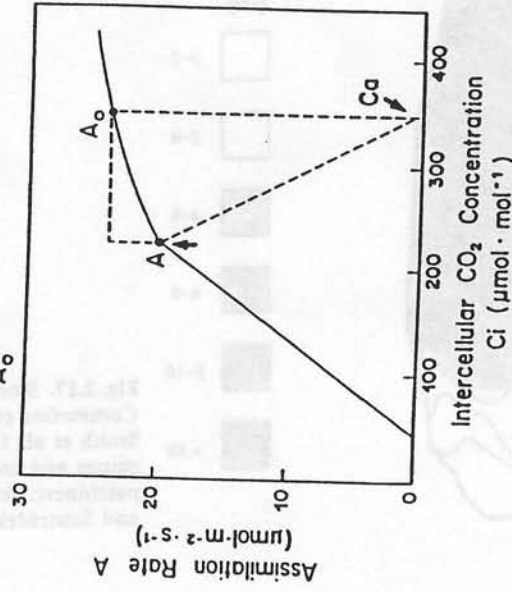
Ziegler, H., C.B. Osmond, W. Stichler, and P. Trimborn. 1976. Hydrogen isotope discrimination in higher plants: correlations with photosynthetic pathway and environment. *Oecologia* 128:85-92.

Since 2002 I've found an additional 177 papers on these topics; do a search and check them out!

(FIGURES)



$$L_s = \frac{A_0 - A}{A_0}$$



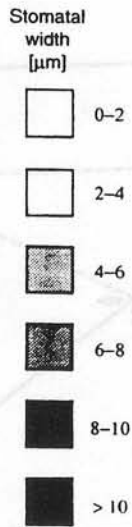


Fig. 2.17. Stomatal patchiness on a leaf of *Commelina communis* at midday. (After Smith et al. 1989). Anatomy, occurrence, causes and consequences of stomatal patchiness: Terashima (1992); Pospíšilová and Šantrůček (1994)

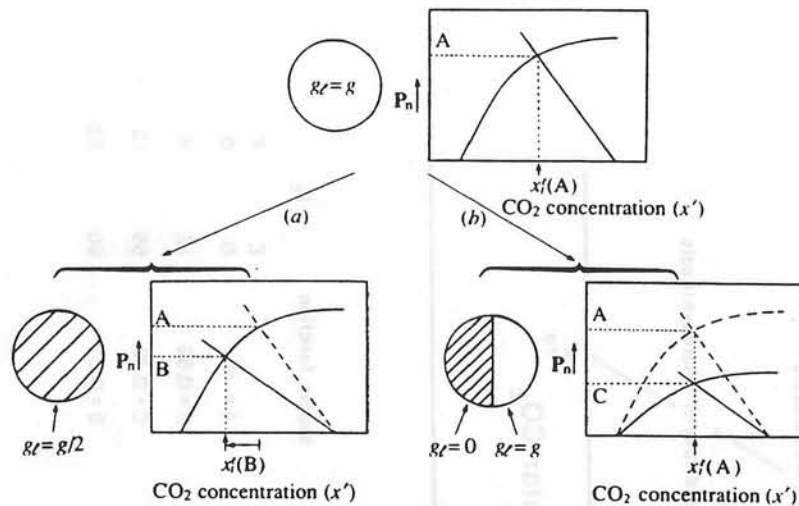
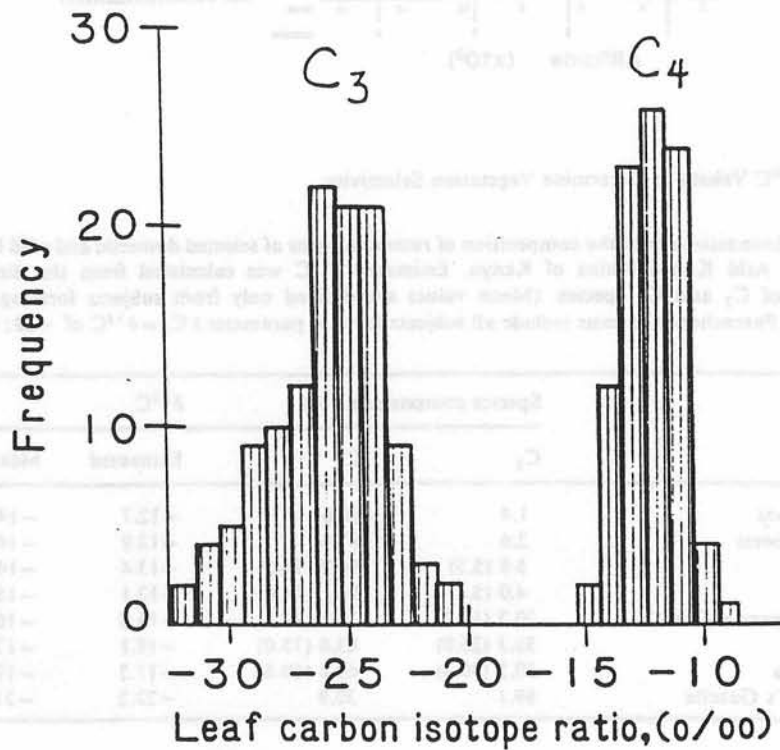
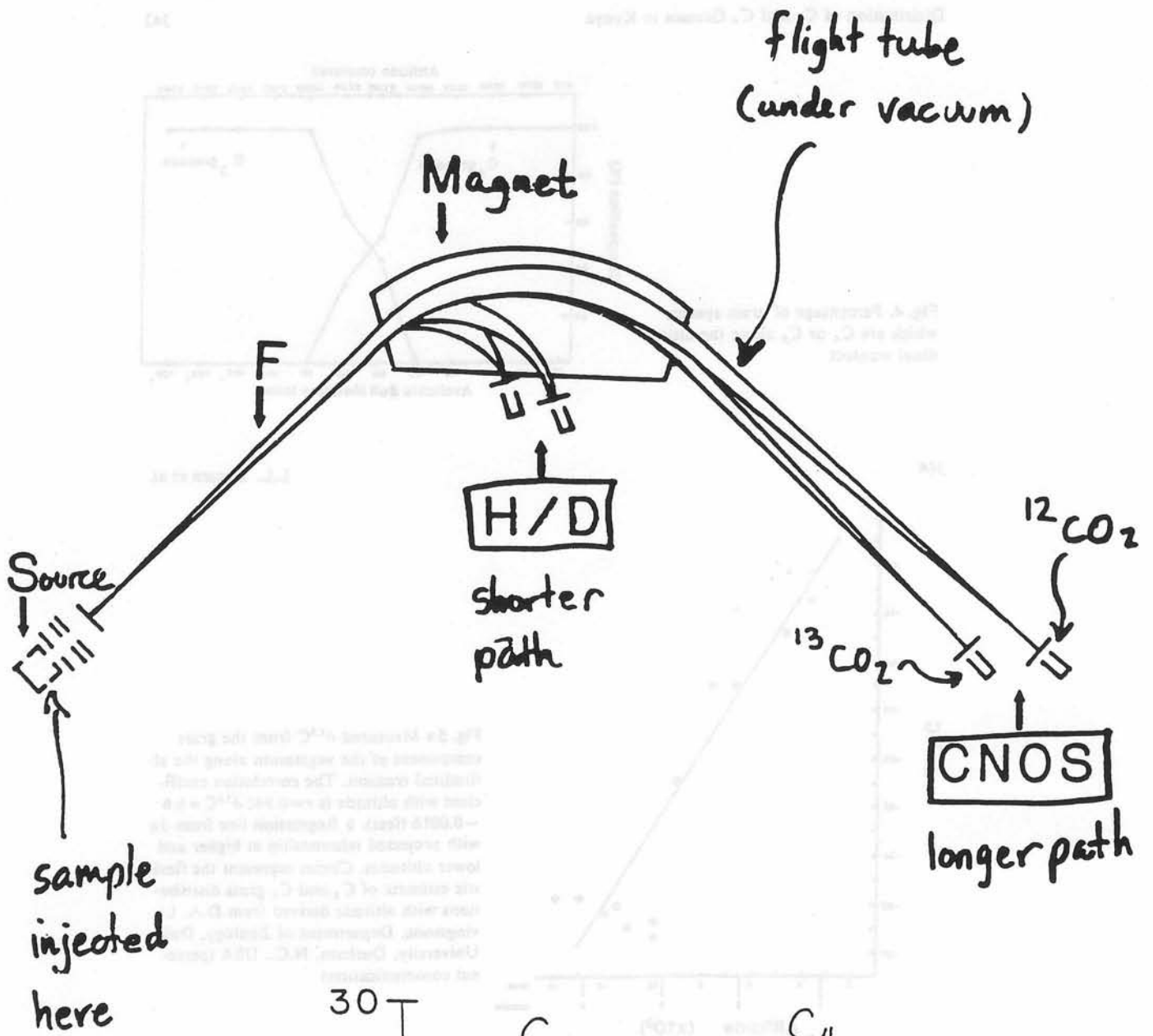


Fig. 7.10. An illustration of the effect of patchy stomatal closure on calculated x'_i and on the estimated mesophyll properties. The top figure represents a healthy leaf with the photosynthesis demand function given by the solid line, resulting in a photosynthetic rate A. Assuming no change in the photosynthetic properties of the leaf we can compare what happens if (a) all the stomata close by 50%, or (b) if half the stomata (in discrete patches unconnected via the intercellular spaces) close completely. In (a) stomatal conductance per unit area falls to $g/2$, assimilation decreases according to the response curve, falling to B ($> A/2$), and the calculated x'_i decreases significantly (implying increased stomatal control of photosynthesis as one would expect). In (b) on the other hand, although the average stomatal conductance over the large area also falls to $g/2$, assimilation would now decrease to C ($= A/2$) as only half the area is now photosynthesising, but because both A and g_r have changed similarly it follows from equation 7.20 that x'_i is apparently unchanged. In addition there is a large apparent shift in the photosynthetic demand function with the photosynthetic capacity apparently reduced by 50% even though there is no real change in the mesophyll properties.



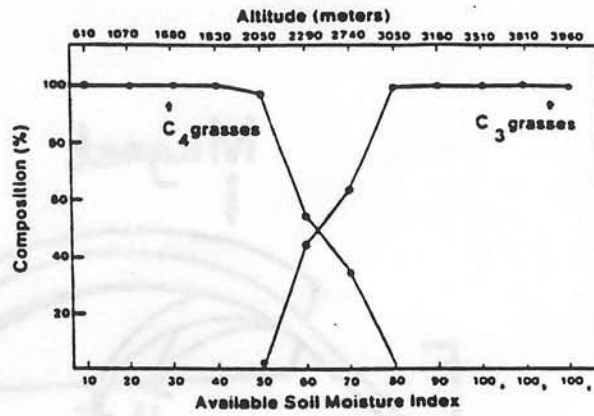


Fig. 4. Percentage of grass species which are C₄ or C₃ along the altitudinal transect

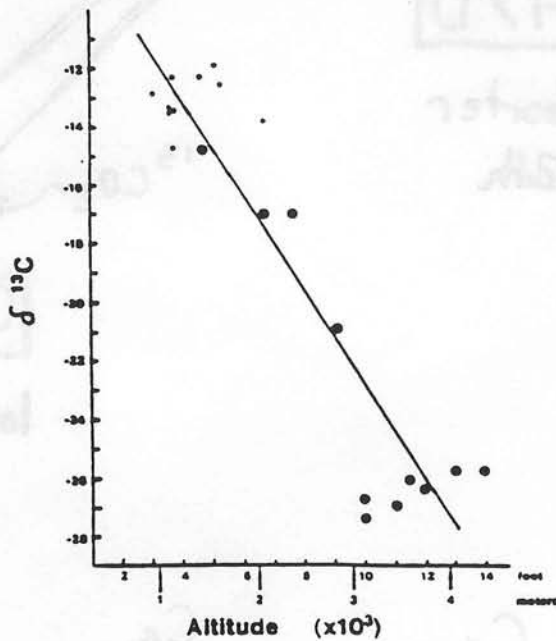


Fig. 5a Measured $\delta^{13}\text{C}$ from the grass component of the vegetation along the altitudinal transect. The correlation coefficient with altitude is $r=0.94$; $\delta^{13}\text{C} = 6.6 - 0.0016$ (feet). b Regression line from 5a with projected relationship at higher and lower altitudes. Circles represent the floristic estimate of C₃ and C₄ grass distributions with altitude derived from D.A. Livingstone, Department of Zoology, Duke University, Durham, N.C., USA (personal communication)

Use of $\delta^{13}\text{C}$ Values to Determine Vegetation Selectivity

Table 2. Mean estimates of the composition of rumen samples of selected domestic and wild herbivores from the Athi Kapiti Plains of Kenya. Estimated $\delta^{13}\text{C}$ was calculated from the direct visual estimate of C₃ and C₄ species. (Mean values are derived only from subjects forming complete data sets. Parenthetical means include all subjects for that parameter.) C₃ = $\delta^{13}\text{C}$ of -28 ; C₄ = $\delta^{13}\text{C}$ of -12.5

Species	Species composition (%)		$\delta^{13}\text{C}$	
	C ₃	C ₄	Estimated	Measured
1. Kongoni	1.4	98.6	-12.7	-14.4
2. Wildebeest	2.6	97.4	-12.9	-14.4
3. Cattle	5.8 (5.5)	94.2 (94.5)	-13.4	-14.6
4. Sheep	4.0 (5.4)	96 (94.6)	-13.1	-15.3
5. Thompson's Gazelle	20.2 (19.2)	79.8 (80.8)	-16.0	-16.9
6. Goat	36.3 (25.0)	63.6 (75.0)	-18.1	-17.7
7. Impala	30.2 (30.4)	69.8 (69.6)	-17.2	-19.0
8. Grant's Gazelle	69.1	30.9	-23.2	-21.9

Table 2. Stable carbon isotope composition of plants in a Georgia old field

Species	$\delta^{13}\text{C}$
C₄ plants:	
<i>Digitaria sp.</i> (crabgrass)	-10.9
<i>Cyperus odoratus</i> (nutgrass)	-10.9
<i>Sorghum halepense</i> (Johnson grass)	-12.1
<i>Euphorbia supina</i> (prostrate spurge)	-12.8
<i>Cynodon dactylon</i> (Bermuda grass)	-12.8
<i>Amaranthus retroflexus</i> or <i>hybridus</i> (pigweed)	-12.9
C₃ plants:	
<i>Cassia obtusifolia</i> (sicklebud)	-27.3
<i>Solidago canadensis</i> (goldenrod)	-27.9
<i>Campsis radicans</i> (trumpet-vine)	-28.0
<i>Lonicera japonica</i> (honeysuckle)	-28.4
<i>Ambrosia artemisiifolia</i> (ragweed)	-28.8
<i>Desmodium tortuosum</i> (beggar's lice)	-28.8
<i>Mollugo verticillata</i> (carpet weed)	-29.1

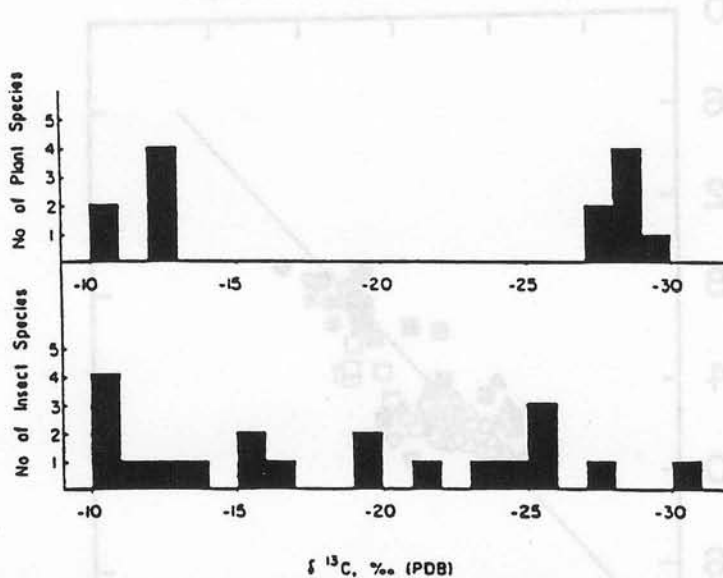
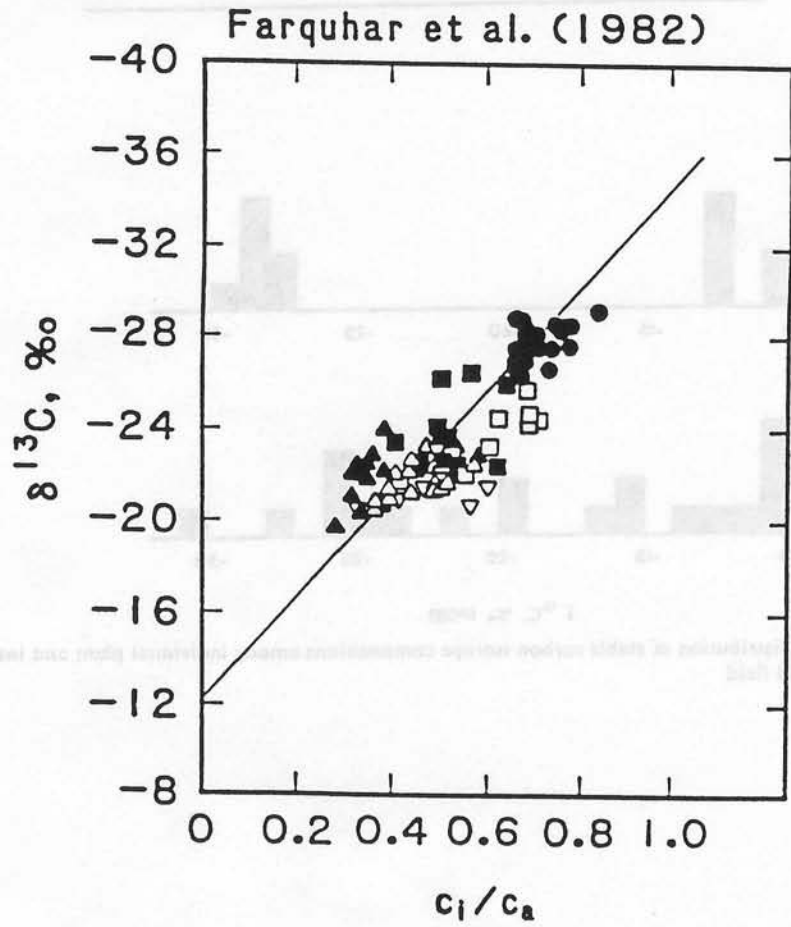
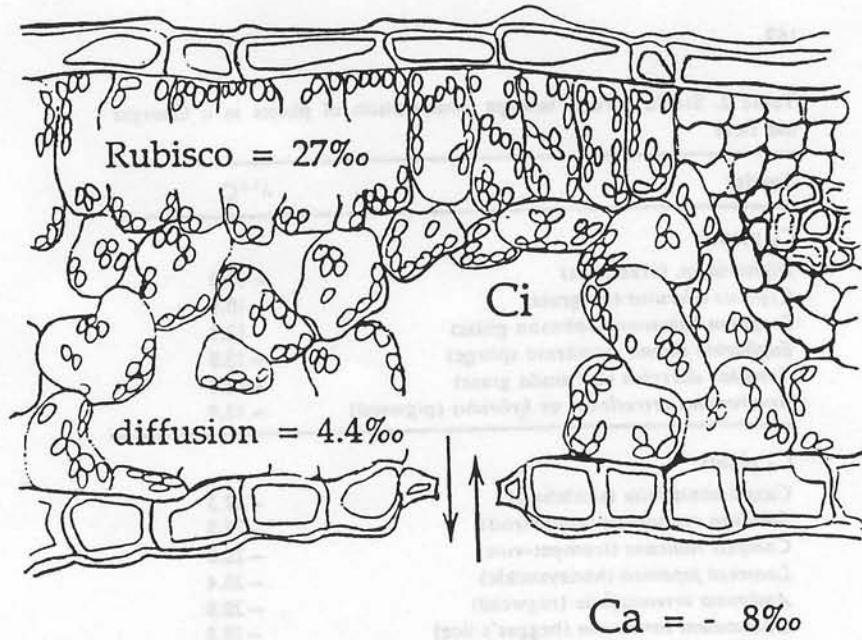


Fig. 1. Distribution of stable carbon isotope compositions among individual plant and insect species in an old field

The relationship between $\delta^{13}\text{C}$ of a photosynthetic leaf and C_i is:

$$\delta^{13}\text{C}_{\text{leaf}} = \delta^{13}\text{C}_{\text{air}} - a - (b - a) \times (C_i/C_a),$$



$$\delta^{13}\text{C} = -12.2 - 22.6 C_i/C_a$$

Table 1 Isotope effects of steps leading to CO₂ fixation in plants.

Process	Isotope effect (α)	Discrimination (‰)	Symbol	Reference
Diffusion of CO ₂ in air through the stomatal pore ^a	1.0044	4.4	<i>a</i>	Craig (16)
Diffusion of CO ₂ in air through the boundary layer to the stomata ^a	1.0029	2.9	<i>a_b</i>	Farquhar (33)
Diffusion of dissolved CO ₂ through water	1.0007	0.7	<i>a_r</i>	O'Leary (98)
Net C ₃ fixation with respect to <i>p_i</i>	1.027	27	<i>b</i>	Farquhar & Richards (39)
Fixation of gaseous CO ₂ by Rubisco from higher plants	1.030 (pH = 8)	30	<i>b_v</i>	Roeske & O'Leary (119) ^b
Fixation of HCO ₃ ⁻ by PEP carboxylase	1.029 (pH = 8.5)	20	<i>b_v</i>	Guy et al (50)
	1.0020	2.0	<i>b_v</i>	O'Leary et al (101)
	1.0020	2.0		Reibach & Benedict (117)
Fixation of gaseous CO ₂ (in equilibrium with HCO ₃ ⁻ at 25°C) by PEP carboxylase	0.9943	-5.7	<i>b_v</i>	Farquhar (33)
Equilibrium hydration of CO ₂ at 25°C	0.991	-9.0	<i>c_v</i>	Emrich et al (31)
	0.991	-9.0		Mook et al (91)
Equilibrium dissolution of CO ₂ into water	1.0011	1.1	<i>c_v</i>	Mook et al (91)
	1.0011	1.1		O'Leary (98)

^aTheoretical value

^bData corrected for dissolution of CO₂

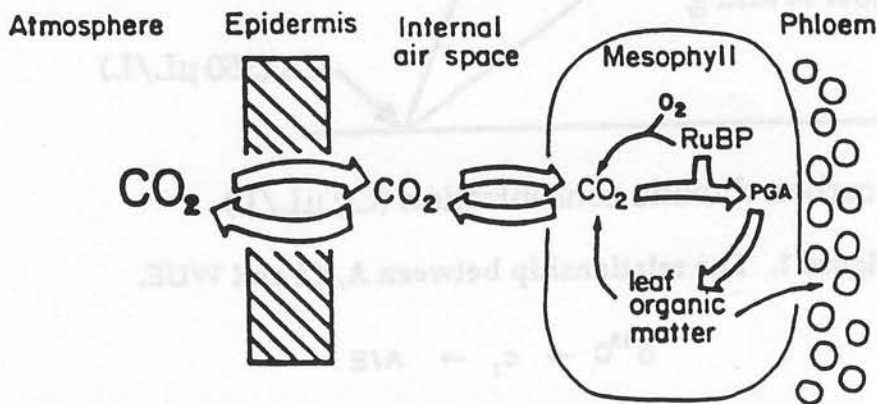


Figure 2. Important steps in CO₂ fixation during C₃ photosynthesis. Sizes of arrows indicate the relative fluxes through the various steps (including the reverse steps) according to the best models available. Sizes of symbols reflect relative concentrations of CO₂ at various stages.

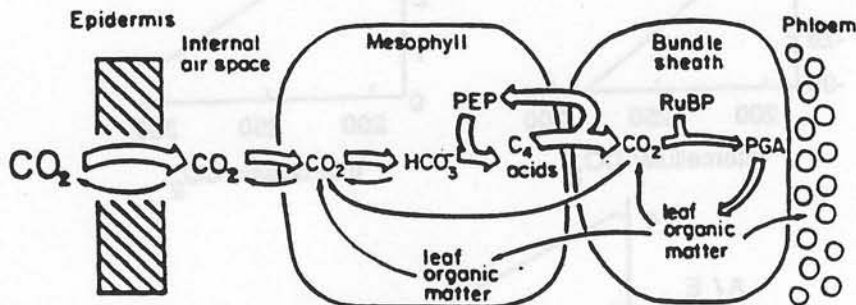


Figure 3. Important steps in CO₂ fixation during C₄ photosynthesis. Sizes of arrows indicate the relative fluxes through the various steps (including the reverse steps) according to the best models available. Sizes of symbols reflect relative concentrations of CO₂ at various stages.

$$A = (c_a - c_i)g / 1.6$$

$$E = \Delta w g$$

$$A/E = \frac{c_a - c_i}{1.6 \Delta w}$$

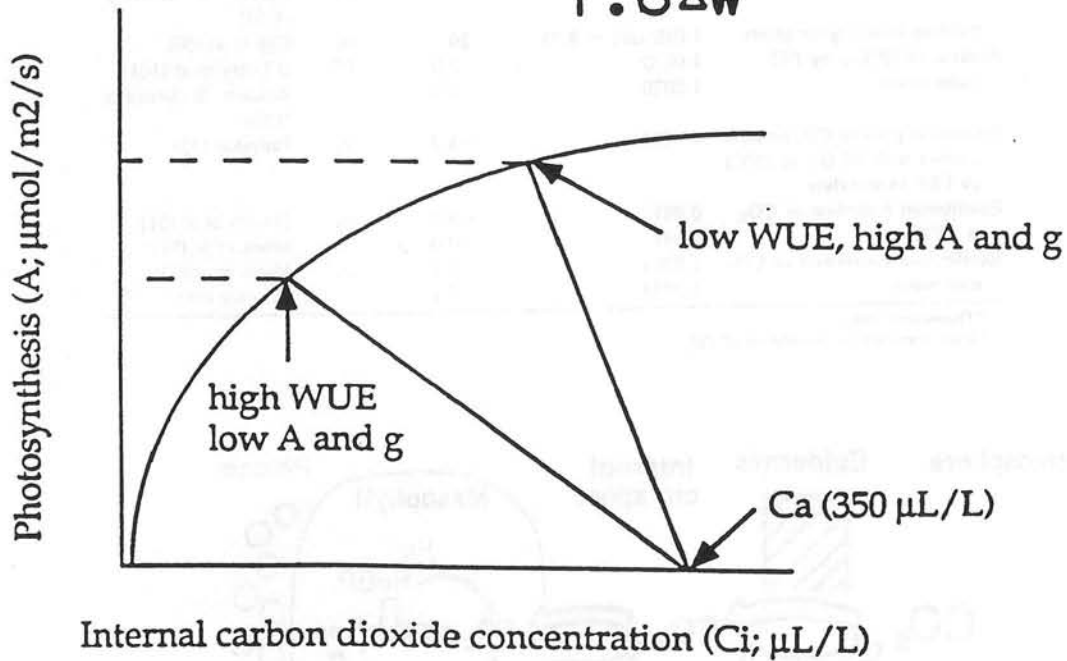
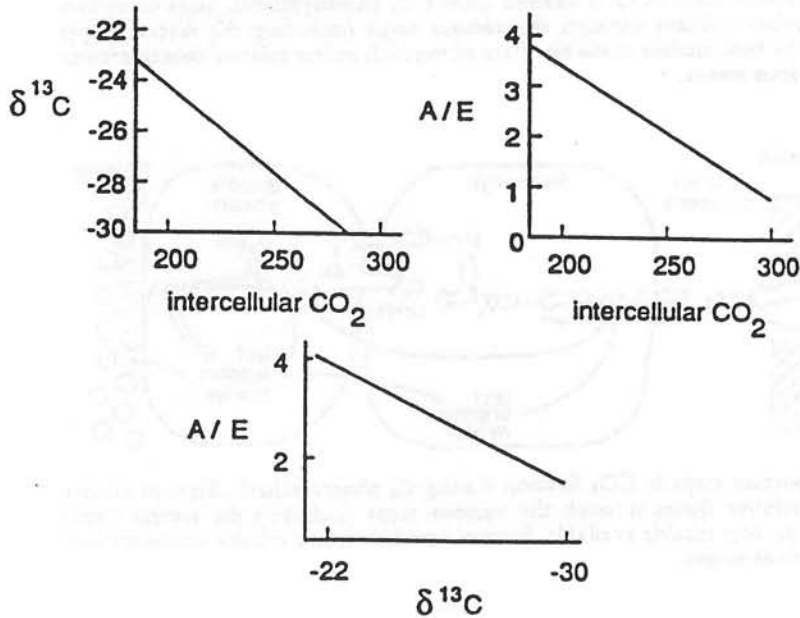
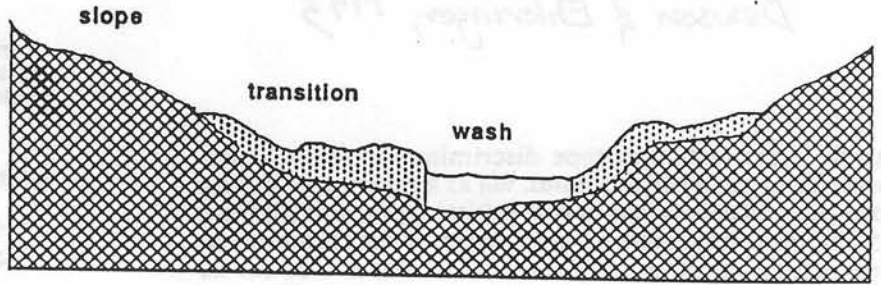


Figure 1. The relationship between A, C_i and WUE.

$$\delta^{13}\text{C} \rightarrow c_i \rightarrow A/E$$



From: Ehleringer & Cooper (1988)



Carbon isotope ratio (o/oo)

	slope	transition	wash
<i>Ambrosia dumosa</i>	-25.4	-26.0	-27.4
<i>Bebbia juncea</i>	-25.8	-26.7	-28.3
<i>Eriogonum inflatum</i>	-25.7	-25.5	-28.2
<i>Larrea divaricata</i>	-22.6	-22.4	-24.1
<i>Porophyllum gracile</i>	-26.6	-27.7	-27.5

Farquhar and Richards (1984)

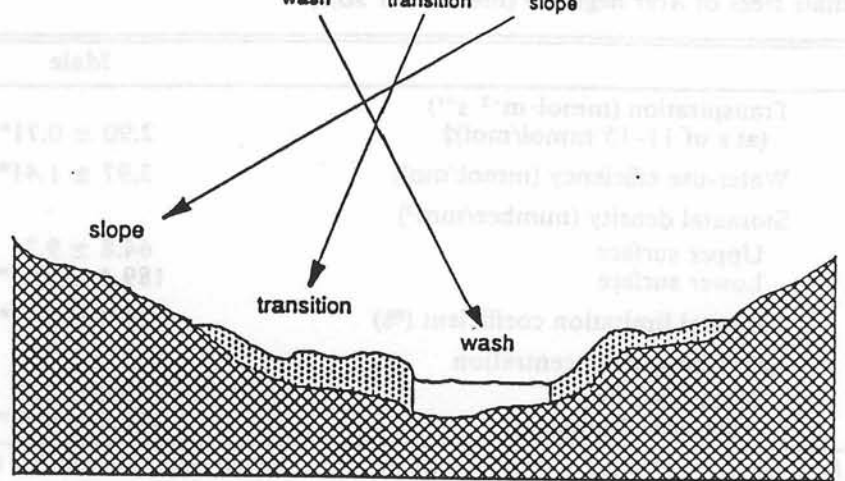
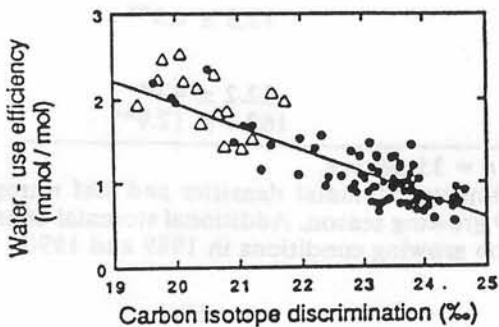
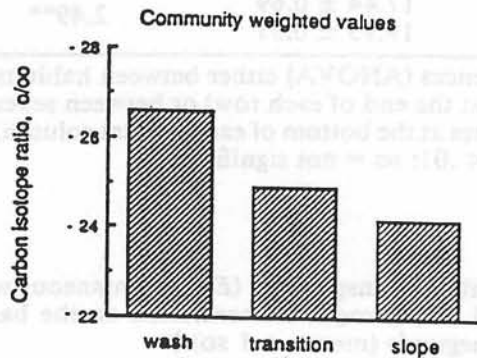
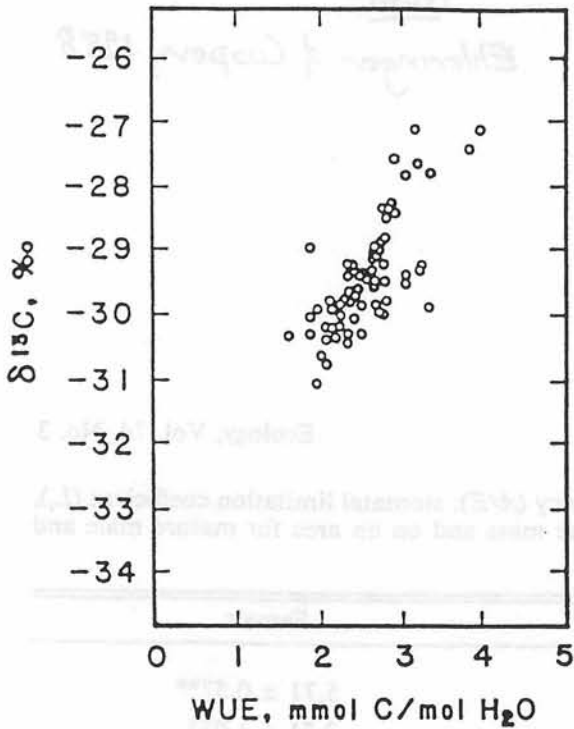


Table 5 & 6 from:
Dawson & Ehleringer, 1993

TABLE 6. Leaf carbon isotope discrimination for *Acer negundo* (Δ , in parts per thousand, ‰) as a function of gender from (A) field collections in two habitat types, and (B) from 1.5-yr-old saplings grown out-of-doors under common environmental conditions. † Values are means \pm 1 SD for 25 separate individuals per sex within each habitat from the field and 12 separate individual saplings of each sex for plants grown in the common environment.

	Habitat		Δ between habitats
	Streamside	Non- streamside	
Male	20.03 \pm 0.35	18.62 \pm 0.64	1.41*
Female	25.51 \pm 0.11	21.67 \pm 0.17	0.16NS
Δ between genders within a habitat	1.48*	3.05**	
B) Common environment collections			
			Δ between genders
Male	17.44 \pm 0.69		2.49**
Female	19.93 \pm 0.51		

† Significant differences (ANOVA) either between habitats within a sex (values at the end of each row) or between sexes within a habitat (values at the bottom of each habitat column) were * $P < .05$; ** $P < .01$; NS = not significant.

Carbon isotope ratio of common shrubs at Oatman, Arizona - plants from wash transition habitat sampled after summer rains

long - lived shrubs	
Larrea divaricata	-24.1
Lycium andersonii	-25.3
medium - lived	
Ambrosia dumosa	-27.4
Encelia farinosa	-26.3
Hymenoclea salsola	-26.5
short - lived shrubs	
Bebbia juncea	-28.3
Eriogonum inflatum	-28.2

↑
from:
Ehleringer & Cooper, 1988

Ecology, Vol. 74, No. 3

TABLE 5. Maximum rate of transpiration (E), instantaneous water-use efficiency (A/E), stomatal limitation coefficient (L_s), stomatal density, and leaf nitrogen concentration on the bases of leaf-tissue mass and on an area for mature male and female trees of *Acer negundo* (means \pm 1 SD). †

	Male	Female
Transpiration ($\text{mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) (at ν of 11-15 mmol/mol) ‡	2.90 \pm 0.71**	5.71 \pm 0.57**
Water-use efficiency (mmol/mol)	3.97 \pm 1.41*	2.71 \pm 1.07*
Stomatal density (number/ mm^2)		
Upper surface	64.8 \pm 9.3	59.1 \pm 12.6
Lower surface	189.4 \pm 23.7**	255.1 \pm 33.9**
Stomatal limitation coefficient (%)	21.2 \pm 5.7**	13.3 \pm 6.9**
Leaf nitrogen concentration		
Mass basis (mg/g)	26.8 \pm 2.8*	32.2 \pm 4.9*
Area basis (mmol/m)	126.9 \pm 9.6**	162.7 \pm 12.9**

* $P < .05$ or ** $P < .01$ (comparisons between the genders, Student's t test); $n = 35$ per sex.

† A , E , A/E , and L_s were determined from laboratory gas exchange experiments. Stomatal densities and leaf nitrogen concentrations were determined on leaves collected in the field during the 1989 growing season. Additional stomatal density information was obtained from plants grown in pot experiments under common growing conditions in 1989 and 1990.

‡ ν = leaf-air vapor pressure gradient; see Eq. 3.

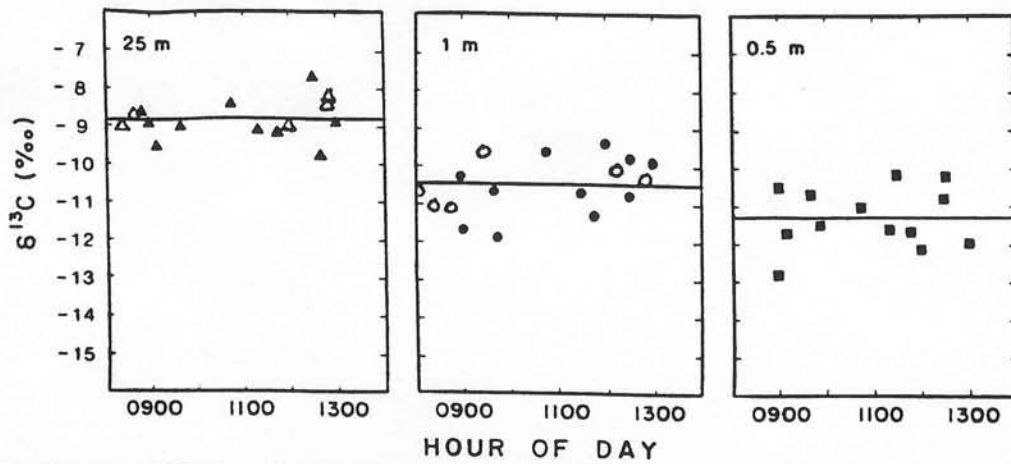


FIG. 1. $\delta^{13}\text{C}$ values of CO_2 from 0800 to 1300 in forest air at three different heights for all plots. Symbols are samples taken at 25 m (Δ , \blacktriangle), 1 m (\circ , \bullet), and at 0.5 m (\blacksquare). Open symbols represent samples taken during the dry season, and closed symbols represent samples taken during the wet season. $N = 15, 18,$ and 13 for 25, 1, and 0.5 m, respectively.

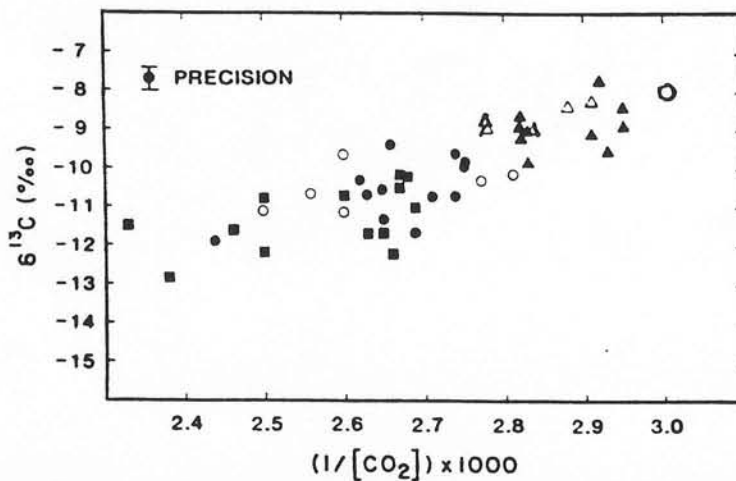


FIG. 2. Relationship between $\delta^{13}\text{C}$ values of forest air as a function of the inverse of CO_2 concentration ($\mu\text{L/L}$). Symbols are described in Fig. 1. Statistics are given in Table 1. Precision (SD) is $\pm 0.3\text{‰}$. The $\delta^{13}\text{C}$ value and concentration of atmospheric CO_2 is shown by the open hexagon in the upper right of the figure.

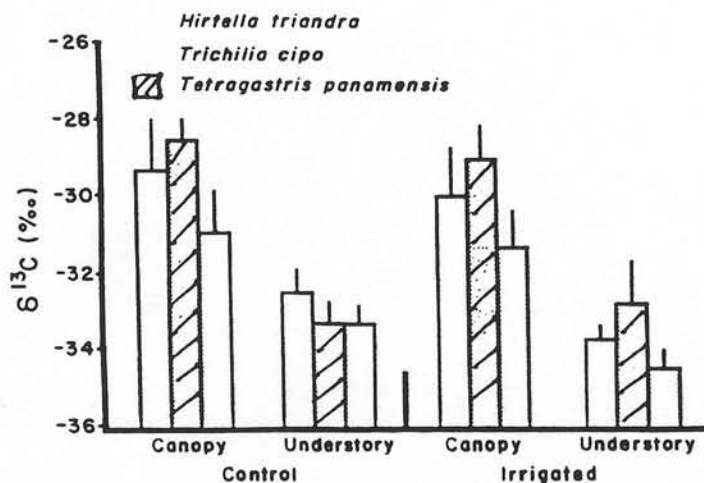


FIG. 3. Variation in $\delta^{13}\text{C}$ values between irrigation treatments and understory and canopy individuals of *Hirtella*, *Tetragastris*, and *Trichilia*. For each species, understory and canopy individuals are significantly different as determined by a mixed model analysis of variance on ranks of the data ($F = 63.1, 84.7,$ and 65.0 ; $df = 1, 21, 1, 24,$ and $1, 20,$ respectively; $P < .0001$ for each species). Error bars are 2 SE. Statistics for treatment effects are given in Results and Discussion: Isotopic Analysis of Plant Material.