From Field et al. (1989) *In* Pearcy et al.

Fig. 11.1 Simplified schematic illustrating how photosynthesis is measured by three basic types of gas-exchange systems. Rates of gas exchange in all systems are determined by mass balance. The calculation of photosynthesis in a closed system is based on the rate of change of CO₂ concentration. Note that a closed system utilizes an absolute, rather than a differential, infrared gas analyzer (IRGA) and requires no flow-measuring device. A differential system calculates photosynthesis from the CO₂ depletion that occurs as air flows at a known rate past a photosynthesizing leaf. In a compensating system, CO₂ depletion by photosynthesis is compensated by CO₂ injection so that the CO₂ concentration in the air exiting the chamber is the same as that in the air stream entering the chamber.

Fig. 11.6 Schematic diagrams of three types of leaf chambers. (a) A controlled-environment chamber similar to that used with the mobile laboratory of Mooney et al. (1971). Circulating water provides the temperature control. Most chambers of this design are made from nickel-plated brass and incorporate a glass window. Even though a large fan blows air directly against the leaf, boundary layer conductances in chambers like this are usually lower than in chambers like those shown in (b) and (c). (b) A controlled-environment chamber similar to that used in the portable system of Field et al. (1982). Temperature in this chamber is Peltier controlled, and circulating water acts as a heat sink or source for the Peltier modules. Most implementations of this design are nickel-plated brass or aluminum with a glass window. (c) One of the chambers (this is the 1 liter version) for use with the ambient-sampling LI-COR LI-6200. This chamber is not actively temperature controlled, but it is made from polycarbonate, a material that transmits much of the thermal infrared radiation emitted by the leaf and other chamber parts. The chamber is lined with a Teflon film to minimize CO₂ and H₂O exchange by chamber materials. This chamber includes constant-area inserts that automatically restrict sampling to a fixed leaf area, eliminating the need for measuring leaf area.
From Field et al. (1989) In Pearcy et al.

Fig. 11.2 Infrared absorption spectra of CO₂ and H₂O (modified from Sesták et al. (1971)).

From LiCor brochure for LI-7000 CO₂ / H₂O Analyzer

The infrared source provides radiation through a single optical path, which is directed to CO₂ and H₂O detectors. Internal chemicals keep the detector and chopper housings free of CO₂ and H₂O vapor.

Fig. 11.3 Three types of IRGA designs. Absolute IRGAs (with sealed reference cells) are shown. The only difference in a differential instrument is that the reference gas may have some CO₂ concentration other than zero and typically flows through the reference cell. (a) An IRGA with a 'Luft' detector and chambers in parallel. (b) An IRGA with a mass flow detector (e.g. Binos). (c) An IRGA that substitutes multiple IR sources, energized at different times, for a mechanical radiation chopper (e.g. Liston-Edwards). In each instrument, the numbers indicate these components: (1) infrared radiation sources (1a and 1b indicate multiple sources), (2) detector, (3) reference cell, (4) sample cell, (5) radiation chopper [in (c), the two sources act as a chopper], (6) gas-filled filters. In (a), CO₂ concentration is calculated from the distension of the flexible membrane in the detector. In (b), the signal is the mass flow of gas between the two chambers of the detector. The detector in (c) is functionally similar to that in (a).
Theoretical equations for Net Assimilation ($A_{net}$) and for Transpiration ($E$):

$$A_{net} = g_{c,{[bl,s]}} \ast (c_a - c_i)$$

$$E = g_{w,{[c,bl,s]}} \ast (w_i - w_a)$$

where:

$g_{c,{[bl,s]}} = $ conductance of CO$_2$ across boundary layer and stomata, respectively

c$_a$ and c$_i$ = CO$_2$ contents of air in atmosphere and in intercellular airspaces, respectively

$g_{w,{[c,bl,s]}} = $ conductance of water vapor across cuticle, boundary layer, and stomata, respectively

w$_i$ and w$_a$ = water vapor contents of air in atmosphere and in intercellular airspaces, respectively
Combining (B1) and (B11) one obtains

\[ E = \frac{u_c (w_w - w_e)}{s (1 - w_e)} \]  

(B5)

The term \( w_e \) in the denominator is neglected in most studies, leading to a slight underestimate of transpiration rate. Typically the outgoing vapour pressure is 20 to 30 mbar, and so at a total pressure of 1 bar, \( w_e \) is between 0.02 and 0.03 and therefore the error is 2-3%.

Assimilation rate is derived as

\[ A = \frac{u_c (1 - w_e)}{s (1 - w_e)} \]  

(B6)

from Eqs. (B2) and (B11).

The IRGAs measure molar densities of \( \text{CO}_2 \). Since the temperature in the cells is constant and the pressure is measured the difference \( A = (c_i - c_o) \) can be determined. Substitution of \( c_o = (c_e - A) \) yields

\[ A = \frac{u_c (1 - w_e)}{s (1 - w_e)} E - c_e \]  

(B7)

The calculation of assimilation rate is influenced by the transpiration rate and estimates are less than those obtained when humidification of the outgoing airstream is not considered. In some experiments presented here the IRGAs were not fitted with water vapour interference filters, and ice traps were used to reduce water vapour concentration in the airstreams entering the IRGAs to a standard magnitude. The effect of humidification on the estimation of \( \text{CO}_2 \) assimilation rate is then eliminated. However, the effect of water condensation at the ice traps needs to be considered. If \( w_{\text{ice}} \) is the flow rate at the ice traps and \( w_{w} \) the mole fraction of water vapour at ice point

\[ A = u_c (\frac{(1 - w_{w})}{s (1 - w_{w})} A) \]  

(B8)

Calculations of total conductance to water vapour and intercellular \( \text{CO}_2 \) concentration. The total conductance to water vapour and the intercellular \( \text{CO}_2 \) concentration were calculated by combining equations derived byJarman (1974), with those derived by Cowan (1977). Jarman considered a ternary system of the gases (vapour, \( \text{CO}_2 \), and air) where there is no molar flux of air. This takes into account not only collisions between water vapour and air, and \( \text{CO}_2 \) and air, but also the collisions between \( \text{CO}_2 \) and water vapour. His general equations, using our notation, were

\[ (w_i - w_e) = \frac{(\bar{A} + \bar{E})}{s_{\text{w}} + s_{\text{w}}} E + \frac{\bar{E}}{s_{\text{w}}} A \]  

(B9)

\[ (c_i - c_e) = \frac{(\bar{A} + \bar{E})}{s_{\text{w}} + s_{\text{w}}} A - \frac{E}{s_{\text{w}}} \]  

(B10)

where \( \bar{w} = (w_i + w_e)/2 \) and \( \bar{e} = (c_i + c_e)/2 \); \( w_i \) and \( c_i \) are the mol fractions of water vapour and \( \text{CO}_2 \) inside the leaf; \( w_e \) and \( c_e \) are the mol fractions of water and \( \text{CO}_2 \) in the surrounding air; \( s \) is the mol fraction of gases other than \( \text{CO}_2 \) and water vapour so that \( \bar{w} + \bar{e} + s = 1 \).

\[ s_{\text{w}} = \frac{CD_{\text{w}}}{1} \quad s_{\text{w}} \quad \frac{CD_{\text{w}}}{1} \quad \frac{CD_{\text{w}}}{1} \]  

(B11)

are the conductances of water vapour in air, \( \text{CO}_2 \) in air and \( \text{CO}_2 \) in water vapour (mol m$^{-2}$ s$^{-1}$), and \( D_{\text{w}} \) and \( D_{\text{w}} \) are the respective binary diffusion coefficients, \( C \) is the total concentration of gas (mol m$^{-3}$) and \( t \) the effective length of the pore. Because \( D_{\text{w}} \geq D_{\text{w}} \) and \( D_{\text{w}} \geq D_{\text{w}} \) it follows that \( g_{\text{w}} = g_{\text{w}} \) and \( g_{\text{w}} = 1.6g_{\text{w}} \).

The first term in Eq. (B9) and (B10) arises from the collisions of water and air, and \( \text{CO}_2 \) and air respectively. The second and third terms in both equations are due to the collisions between water vapour and \( \text{CO}_2 \). In Eq. (B9) these two terms are negligible because the mole fraction of \( \text{CO}_2 \) is a small part of the total gas mixture and the flux of \( \text{CO}_2 \) is small compared to the flux of water (\( A \neq E \)). Equation (B9) therefore simplifies to

\[ (w_i - w_e) = \frac{(1 - \bar{w})}{s_{\text{w}}} E \]  

(B12)

or rearranging.

\[ E = \frac{g_{\text{w}} (w_i - w_e) + \bar{w} E}{s_{\text{w}}} \]  

(B13)

This equation can also be derived considering a binary system of the gases, water and air. It differs from the incorrect version commonly employed by the term \( \bar{w} E \), which typically accounts for 3% of the estimate of \( E \).

The total conductance to water vapour is obtained from Eq. (B13) as

\[ \frac{E (1 - \bar{w})}{s_{\text{w}} (w_i - w_e)} E \]  

(B14)

The boundary layer conductance to water vapour, \( g_{\text{w}} \) for each side of the leaf chamber was 0.4 mol m$^{-2}$ s$^{-1}$. Stomatal conductance to water vapour, \( g_{\text{w}} \) is calculated from

\[ \frac{1}{g_{\text{w}}} = \frac{1 + 1}{g_{\text{w}}} + \frac{1}{g_{\text{w}}} \]  

(B15)

and the total conductance to \( \text{CO}_2 \), \( g_{\text{w}} \) is then obtained from

\[ \frac{1}{g_{\text{w}}} = \frac{1 + 1}{g_{\text{w}}} + \frac{1}{g_{\text{w}}} \]  

(B16)

Stomatal conductance to \( \text{CO}_2 \) \( g_{\text{w}} = g_{\text{w}}/1.6 \) and boundary layer conductance to \( \text{CO}_2 \) \( g_{\text{w}} = g_{\text{w}}/1.37 \), where 1.6 is the ratio of diffusivities of \( \text{CO}_2 \) and water in air, 1.37 is the ratio of diffusivities of \( \text{CO}_2 \) and water vapour in the boundary layer (the latter value results from the Puchalski analysis of mass transfer from a plate in laminar parallel flows (Kays 1966)).

As \( \bar{w} + \bar{w} = 1 \) and \( s_{\text{w}} = g_{\text{w}} \) Eq. (B16) simplifies to

\[ A = g_{\text{w}} (c_i - c_e) - \bar{E} \]  

(B17)

Here the interaction between water and \( \text{CO}_2 \) is important since the molar flux of water out of the stomatal pore is considerably larger than that of \( \text{CO}_2 \) into the pore.

Intercellular \( \text{CO}_2 \) is then calculated from Eq. (B17) as

\[ \bar{c}_i = \left( \frac{g_{\text{w}}}{g_{\text{w}} + \frac{E}{2}} \right) \bar{c}_e \]  

(B18)

The intercellular \( p(\text{CO}_2) \) is then given by the product of \( c_i \) and the total pressure.

The rates of assimilation and transpiration, conductance, and intercellular \( p(\text{CO}_2) \) were calculated independently for each surface of the leaf. The sum of assimilation rate, transpiration rate and conductance are expressed in terms of leaf area of one side of the leaf only. Intercellular \( \text{CO}_2 \) appropriate to the whole leaf is calculated as in Eq. (B18) using the summed values for \( A \), \( E \) and \( s_{\text{w}} \).

We wish to thank Professor I.R. Cowan and Dr. T.J. Andrews for discussion relating to the calculation of gas exchange parameters,
Fig. 2.2. Schematic of an isotope ratio mass spectrometer used to make isotope determinations. In the source region, gas molecules are ionized as they encounter electrons boiling off a hot filament. The charged ions are accelerated via electric fields through a stainless steel flight tube (not shown) maintained under vacuum. In the central magnetic field, charged ions are separated according to inertia, and dispersed towards collectors for automated counting by computers. Due to their small masses and consequent low inertia, the hydrogen ion beams are sharply bent by magnet focusing, while magnet focusing results in much more gradual bends in flight paths of the ion beam for gases with higher masses, especially CO$_2$, N$_2$, O$_2$ and SO$_2$.

**TABLE 2.1. Isotope Compositions of International Reference Standards.**

<table>
<thead>
<tr>
<th>Ratio, H/L$^a$</th>
<th>Value, H/L$^a$</th>
<th>% H</th>
<th>% L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard Mean Ocean Water (SMOW)</td>
<td>$^3$H/$^1$H</td>
<td>0.00015576</td>
<td>0.015574</td>
</tr>
<tr>
<td></td>
<td>$^1$O/$^1$H</td>
<td>0.0037399</td>
<td>0.037900</td>
</tr>
<tr>
<td></td>
<td>$^{18}$O/$^{16}$O</td>
<td>0.0020952</td>
<td>0.020952</td>
</tr>
<tr>
<td>Pee Dee Belemnite (PDB)</td>
<td>$^{13}$C/$^{12}$C</td>
<td>0.011180</td>
<td>1.0286</td>
</tr>
<tr>
<td>and Vienna-PDB (VPDB)</td>
<td>$^{13}$O/$^{16}$O</td>
<td>0.0035859</td>
<td>0.0385</td>
</tr>
<tr>
<td></td>
<td>$^{18}$O/$^{16}$O</td>
<td>0.0020972</td>
<td>0.020952</td>
</tr>
<tr>
<td>Air (AIR)</td>
<td>$^{15}$N/$^{14}$N</td>
<td>0.0036765</td>
<td>0.3636</td>
</tr>
<tr>
<td>Canyon Diablo Troilitie (CDT)</td>
<td>$^{35}$S/$^{32}$S</td>
<td>0.0077522</td>
<td>0.74865</td>
</tr>
<tr>
<td>and Vienna-Canyon Diablo</td>
<td>$^{34}$S/$^{32}$S</td>
<td>0.0044162</td>
<td>4.19719</td>
</tr>
<tr>
<td>Troilitie (VCVD)</td>
<td>$^{36}$S/$^{32}$S</td>
<td>0.0001533</td>
<td>0.01459</td>
</tr>
</tbody>
</table>

$^a$H and L indicate heavy and light isotope components, respectively.

**Source:** Ratio values are taken from Hayes (2002) for H, C, N, and O isotopes and from Ding et al. (2001) for S isotopes. Historical values for PDB and newer values for VPDB are considered equivalent (based on data in Coplen 1983, 1996), and similarly, historical values for CDT (Coplen and Krouse 1998) and newer values for VCDT also are considered equivalent (Ding et al. 2001). See Ding et al. (2001) and Hayes (2002) for errors associated with the ratio measurements.
Box 7.2. Isotope Fractionation During Diffusion

Diffusion in a Vacuum

Most of the time, we think of chemical processes fractionating isotopes. But some physical processes also fractionate isotopes. Here is an example. Imagine two stable isotope twins, gases in a vacuum, like bowling balls. You give each a push with the same force. What will happen?

Let's think of CO₂ gas with twins ¹²CO₂ and ¹³CO₂ of masses 44 and 45 that are, respectively, "light" and "heavy." If we push with equal force, \( F = \frac{1}{2} m v^2 \) where \( v \) is the velocity, then

\[
F = \frac{1}{2} (44) v_{\text{light}}^2 = \frac{1}{2} (45) v_{\text{heavy}}^2,
\]

and we can calculate the ratio of the velocities (although we don’t calculate the actual velocities in this example), \( v_{\text{light}}/v_{\text{heavy}} = \sqrt{45/44} = 1.0113 \) 1.0113.

This result is that the light CO₂ will travel 1.13% (11.3‰) faster than the heavy CO₂ molecule. This is fractionation in action.

If you do this calculation for hydrogen that has two stable isotopes (¹H = protium and ²H = deuterium, or D), you find that H₂ (mass 2) gas travels 22.47% (224.7‰) faster than HD (mass 3) gas. Because the difference between protium and deuterium is very large (2x), most hydrogen isotope fractionations are larger and more obvious.

You might think that physical separations of isotopes are rare, but you would be wrong. They are the central principle used in mass spectrometers, our main instruments for measuring isotope values (see Figure 2.2 in Chapter 2).

Diffusion in Air and Water

Collisions affect the diffusional separation of isotopes. If there are many collisions, the isotope differences are dampened. In a vacuum, there are minimal collisions, so the physical fractionations are maximal. At the other extreme, in liquids, molecules collide so frequently with water that the mass differences of the isotopes don’t make a difference. Solvation quenches the physical isotope effects. But what about diffusion in air?

The effect in air is given by a formidable equation involving “reduced mass ratios” (the square root term):

\[
D_1/D_2 = \sqrt{\frac{[C_2 + C']}{[C_1 + C']}},
\]

where \( D_1/D_2 \) is the ratio of diffusion coefficients, \( C \) and \( M \) refer to the concentrations and molecular weights of the two isotope molecules 1 & 2, and the primed (') terms refer to \( C \) and \( M \) in air (Geochimica et Cosmochimica Acta 3, p. 73, 1953). Solving this equation for diffusion of light (¹²CO₂) and heavy (¹³CO₂) carbon dioxide in air, one obtains 4.4% faster diffusion of light CO₂ than heavy CO₂ (Phytochemistry 20:553–556; 1981), a much reduced effect versus the 11.3‰ effect calculated above for diffusion in a vacuum. Collisions make the difference.

In most biological systems, the presence of abundant water ensures many collisions. This makes physical fractionation effects during diffusion small and near zero. But when gases diffuse in air, such as entry of CO₂ into plant stomata during photosynthesis, diffusion effects can be important. These diffusion effects are included in photosynthesis models described in Section 7.7.
Box 7.3. Kinetic Isotope Effects (KIE) and Equilibrium Isotope Effects (EIE), Carbon Isotope Examples

For most chemical reactions, the light isotope molecules react faster than heavy isotope molecules. If the rates of reaction are kinetic $k$ rates, then the reaction rates for molecules with light and heavy carbon isotopes, $^{12}$C and $^{13}$C, can be abbreviated as $^{12}k$ and $^{13}k$. The kinetic isotope effect (KIE) is the contrast in the rate constants $\alpha$, where $\alpha = \frac{^{13}k}{^{12}k}$ and $\Delta$ is the permil fractionation factor derived from $\alpha; \Delta = (\alpha - 1) \times 1000$. Most biological reactions involve KIEs and show no or weak dependence on temperature.

An equilibrium isotope effect (EIE) is the net sum of two opposing kinetic isotope effects that apply in an exchange reaction. In these two-way reactions, the heavy isotope concentrates where it is bound most strongly. In these reactions, this equilibrium fractionation is:

$$\alpha_{eq} = \frac{R_{heavy\ molecule}}{R_{light\ molecule}}$$

where $R$ is the $^{13}$C/$^{12}$C ratio. EIEs often change predictably with temperature.

A Carbon KIE for Photosynthesis

The Rubisco enzyme fixes carbon in plant photosynthesis, adding CO$_2$ to a five-carbon compound to form a six-carbon sugar. The lighter isotope reacts faster in this kinetic reaction with bond formation, and the KIE is $\alpha = 1.029$, or $\Delta = 29\%$. So, if CO$_2$ in air currently has a carbon isotope value of $-8\%$, and conditions allow full expression of the fractionation, the KIE lowers this value to $-37\%$ for added photosynthetic carbon. However, other reactions often partly control the overall kinetics of photosynthesis, so that the final fractionation is usually reduced from $29\%$, to, for example, about $20\%$ for common terrestrial C$_3$ plants.

A Carbon EIE for Atmospheric CO$_2$

Carbon dioxide gas dissolves in water where it can further react with water. Dissolved CO$_2$ hydrates to form carbonic acid that then dissociates to bicarbonate, all in reversible exchange reactions:

$$\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{H}_2\text{CO}_3 \rightarrow \text{H}^+ + \text{HCO}_3^-.$$

In EIE exchange reactions, the rule is that the heavy isotope concentrates where it is most strongly bonded, and because dissolved CO$_2$ is not bound up with water (and is termed “free” CO$_2$), you might correctly guess that the heavy isotope of carbon concentrates in HCO$_3^-$. For this particular exchange at 15°C, $\alpha = 1.009\%$, or $\Delta = 9\%$, with bicarbonate about 9% heavier than dissolved CO$_2$. The CO$_2$–bicarbonate exchange reaction plays out constantly on a global scale between the atmosphere and the ocean, where the ocean has an average bicarbonate value near $+1\%$. Further equilibria between bicarbonate and carbonate occur in seawater, but do little to change this overall fractionation relationship. Atmospheric CO$_2$ has a $\delta^{13}$C value of $-8\%$, 9% lower than that of bicarbonate due to equilibrium exchange. This CO$_2$–bicarbonate exchange thus largely controls the $-8\%$ isotope value of atmospheric CO$_2$ used by plants in the KIE above.

\[ \text{Fig. 2. Relationship between isotopic discrimination, } \Delta, \text{ in leaf material and WUE in each treatment in the winter experiment. The correlation coefficients are: } S, 0.86 \ (P < 0.01); S,J, 0.48 \ (P < 0.06); J, 0.72 \ (P < 0.01); A+7d, 0.24. \]


Limiting factors

Both W & N  

$+N > \text{control} > +W+N > +W$


\[ \text{Community weighted values} \]

\[ \text{Fig. 1. Cross-sectional depiction of the site, illustrating wash, transition and slope microhabitats} \]

\[ \text{Fig. 2. Mean carbon isotope ratio of the perennial vegetation, weighted for species abundance, in wash, transition and slope microhabitats} \]
Figure 1. Location of mountain ranges used for gas exchange measurements. Mountain ranges with Juniperus occidentalis are indicated by diamonds (●) and those with J. osteosperma are denoted by triangles (▲). Locations of nearest weather stations with long-term records (> 25 years) are indicated by circles (○). Climate diagrams (source: Walter et al. 1973) have one line and left y-axis for mean monthly temperature, the other line and right y-axis for mean monthly precipitation, area shaded with vertical lines representing periods during the year when precipitation is sufficient for plants, and solid area representing periods when water deficits occur.

Figure 2. Relationships between δ¹³C from leaf cellulose with (A) precipitation for the period of October 1994–September 1995 and with (B) plot elevation, and (C) relationship between observed carbon isotope discrimination (Δ) and the ratio of the partial pressures of CO₂ in subambient cavities and in ambient air (p/ pₐ) integrated over the spring–fall 1995 growing season for each individual tree. First-order regression lines are the solid lines in (A) and (C); dashed line in (C) is a simplified theoretical relationship. Adjusted R² for the regression between variables are given. Ranges with J. occidentalis are indicated by circles; J. osteosperma by triangles; low altitude plots by open symbols; and high altitude plots by solid symbols. For (A) and (B), δ¹³C was averaged over all trees on the plot. Error bars are standard errors.
\[ \Delta t - \Delta = \frac{(b-a_t)A}{g_m P_a} \int e^{R_0 k + fT} \]

From Moore et al. (1999) Tree Physiology 19:421-433

Fig. 5.8. Conceptual mixing models for carbon isotopes. A two-source model (Model A) with -20‰ from Source 1 and -10‰ from Source 2 contributing 50/50 to -15‰ (open circles; closed circles are sources). But if there is a third Source 3 with intermediate -15‰ isotope values, this complicated interpretation of the isotope results (model B), creating a "mixing muddle" with no unique solution, i.e., source contributions of 50/0/50 and 0/100/0 were both logically possible.

Fig. 5.17. Mixing models and muddles. Bottom graph shows mixing middle where there are three sources and no unique solution for source contributions to the sample, which is shown as a filled triangle and sources are depicted as squares. To resolve the middle, one can measure another tracer, gaining resolution if lucky (left middle) or not gaining resolution if unlucky (right middle). A sure way to gain resolution is to add isotope artificially to one source (top). All sources contribute equally to the sample in these examples.