Stable Carbon Isotope Geochemistry

Key Reading:

Suggested Reading:

“Classic” papers

Stable Carbon Isotopes

There are 2 stable isotopes of carbon:

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Abundance</th>
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<tbody>
<tr>
<td>$^{12}$C</td>
<td>98.89 %</td>
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<tr>
<td>$^{13}$C</td>
<td>1.11 %</td>
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</table>

- Ratio $^{13}$C/$^{12}$C = 1.225 x 10^{-2} (on average)
- However, this ratio varies slightly among different carbonaceous materials

Nomenclature:

$$ \delta^{13}C \text{ (permil, ‰)} = \left[ \frac{^{13}C/^{12}C_{sample}}{^{13}C/^{12}C_{standard}} - 1 \right] \times 1000 $$

Standard reference materials:

- PeeDee Belemnite (carbonate) $1.123 \times 10^{-2}$ PDB
- Solenhofen limestone $1.1218 \times 10^{-2}$ NBS-20

Notation and nomenclature

- $\delta$ notation
- $\delta$ = 1 ‰
- $\delta^{13}C$ (‰)
- Positive: enriched, heavier, higher
- Negative: depleted, lighter, lower
Processes Controlling Isotope Composition of Sedimentary Organic Matter

Production

Primary production
- Photosynthesis - phytoplankton, higher plants, cyanobacteria.

Secondary production
- Chemoautotrophy - sulfide oxidizers, methanogens.

Recycling

Aerobic recycling
- Respiration - aerobic heterotrophic bacteria
- Methane recapture - methanotrophs

Secondary production
- Fermentation

Isotope fractionation effects

- An isotope effect (a physical phenomenon) leads to fractionation (an observable quantity)

Fractionation factor:
- By convention, the magnitude of the equilibrium isotope effect is expressed as a fractionation factor:
  - e.g. for:
    \[ ^{13}\text{CO}_2(g) + H^{12}\text{CO}_3(aq) = ^{12}\text{CO}_2(g) + H^{13}\text{CO}_3(aq) \]
- The fractionation factor, \( \alpha \), is expressed as:
  \[ \alpha_{\text{HCO}_3^-/\text{CO}_2} = \frac{(^{13}\text{C}/^{12}\text{C})_{\text{HCO}_3^-}}{(^{13}\text{C}/^{12}\text{C})_{\text{CO}_2}} \]

A related expression is the "difference fractionation factor"
\[ \varepsilon \equiv \Delta^{13}\text{C} = \delta^{13}\text{C}_{\text{product}} - \delta^{13}\text{C}_{\text{reactant}} \]

2 types of isotope effect:
(i) Equilibrium isotope effects
(ii) Kinetic isotope effects
**Equilibrium isotope effects**

1. Rule of thumb - the heavy isotope ($^{13}\text{C}$) is concentrated in the chemical compound in which it is bound most strongly.
2. The expression for the isotopic exchange reaction is written just as for any chemical reaction and the equilibrium constant ($K$) is determined in the same way.
   - e.g. for the reaction:
     \[
     ^{13}\text{CO}_2(\text{g}) + \text{H}^{12}\text{CO}_3(\text{aq}) = ^{12}\text{CO}_2(\text{g}) + \text{H}^{13}\text{CO}_3(\text{aq})
     \]
     \[
     K = \frac{[^{12}\text{CO}_2(\text{g})][\text{H}^{13}\text{CO}_3(\text{aq})]}{[^{13}\text{CO}_2(\text{g})][\text{H}^{12}\text{CO}_3(\text{aq})]}
     \]
   - The main equilibrium isotope system affecting organic carbon isotope compositions is the inorganic carbonate buffer system. At seawater pH:
     \[
     \begin{array}{c|ccc|c}
     \text{species} & \Delta ^{13}\text{C} & \text{CO}_2(\text{g}) & \text{CO}_2(\text{aq}) & \text{H}_2\text{CO}_3(\text{aq}) & \text{HCO}_3^- \text{aq} \\
     \hline
     \Delta ^{13}\text{C} & -7 & +1 & +8 & -1 & -1.7 \\
     \end{array}
     \]
   - N.B. The major fractionation effect is the hydration of CO$_2$.
   - (i.e. bicarbonate is enriched in $^{13}\text{C}$ relative to CO$_2$ in solution by ca. 8 $\%$)
   - In equilibrium isotope effects, the difference between the reactant and product depends only on temperature, and not the distribution of material between product and reactant.
   - e.g., while relative abundances of CO$_2$(aq) and HCO$_3^-$ varies as a function of pH, isotope differences only vary with temperature.

**Kinetic Isotope Effects**

- Many reactions involving organic compounds result in kinetic isotope effects.
- The effect results from different rates of conversion of reactants with $^{13}\text{C}$ and $^{12}\text{C}$ to a product. Activation energy for light isotopic species is smaller, and thus in general the species with the lighter isotope will react faster.
- By convention the rate constant for the species with the light isotope is placed as the numerator and almost always the ratio is >1. This is called the standard (or "normal") isotope effect.
- Fractionation factors ($\Delta ^{13}\text{C}$) can be determined as for equilibrium isotope effects.
- Two processes which give rise to kinetic isotope effects:
  - Transport processes
  - Chemical processes

**Kinetic isotope effect terminology:**
- Normal = Light isotopic species reacts more rapidly.
- Inverse = Heavy isotopic species reacts more rapidly.
- Primary = Isotopic substitution at a position to which a chemical bond is made or broken influences the reaction rate.
- Secondary = Isotopic substitution at a remote position influences the reaction rate.
Isotope Fractionation in Biological Processes

Single carbon substrates (CO₂, CH₄)
- Fixation of CO₂ by primary producers (photosynthesis)
- Fixation of CO₂ by chemoautotrophs (sulfide oxidisers, methanogens)
- Processing of intermediates in methanogenesis
- Assimilation of C₁ compounds by methylotrophs

Multi-carbon substrates
- Assimilation of organic molecules by heterotrophic bacteria
- Catabolic metabolism of consumers at all levels
- Biosynthesis in all organisms

Isotope Fractionation during Photosynthesis, εᵣ

In photosynthesis ¹²CO₂ is preferentially taken up relative to ¹³CO₂. There are two stages when kinetic isotope effects can occur:

1. Transport (diffusion) processes
   - Gas phase diffusion (i.e. Atmospheric CO₂ → dissolved CO₂ in leaf)
     Approx. fractionation factor: 4.4 ‰ (i.e., depletion = -4.4 ‰)
     Only important for emergent (vascular) plants where air/leaf interaction occurs.
   - Liquid phase diffusion of CO₂ or HCO₃⁻
     Approx fractionation factor: 0.8 ‰ (relatively minor)

2. Chemical (Enzymatic) processes
   - Four pathways:
     (i) C₃ (Calvin-Benson)
     (ii) C₄ (Hatch-Slack)
     (iii) CAM
     (iv) Bacterial
(i) The C₃ (Calvin-Benson) pathway

- Most common for terrestrial (vascular) plants and phytoplankton (also cyanobacteria).
- All trees use C₃ pathway.

Characteristics:
- optimum growth temperature: 20-35°C
- CO₂ compensation point: 0.004%
- light saturation 3,000 ft.cdl
- max. photosynthetic rate: slow
- enzyme: Ribulose-1,5-biphosphate (RuBP) carboxylase-oxygenase (*RUBISCO*)
  \[ \Delta^{13} \text{CO}_2: -23 \text{ to } -41 \text{ \%o}. \]
  ave. \(-27\) \%o for land plants
  ave. \(-25\) \%o for unicellular phytoplankton

*This difference reflects either differences in carbon transport/fixation mechanisms (see below) or different isotope effect for RUBISCO between emergent and aquatic plants.

Overall reaction:
\[
6 \text{CO}_2 + 12 \text{NADPH} + 18 \text{ATP} \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + 12 \text{NADP}^* + 18 \text{ADP}
\]
(energy and reduction power come from \(h\nu\) splitting of \(\text{H}_2\text{O}\) mediated by chlorophyll)

Carbon isotopic fractionation during C₃ photosynthesis

Model describing the isotopic fractionation, \(\Delta\), in C₃ plants:
\[
\Delta = a + \left(c/c_a\right)(b - a)
\]
where
- \(a\) is the isotope effect associated with diffusion of CO₂ into the plant (~ 0.8 to 4.0 \%o)
- \(b\) is the fractionation associated with carboxylation (by RUBISCO enzyme)
- \(c/c_a\) is the concentration ratio of CO₂ internal to CO₂ external.
- When \(c/c_a = 1\) (i.e. unlimited CO₂) max RUBISCO fractionation, \(b\) expressed.
- When \(c/c_a << 1\) (i.e. limited CO₂) diffusion limited, and only \(a\) expressed.

Formation of 2 molecules of 3-phosphoglycerate from ribulose 1,5-bisphosphate

\[\text{RuBP (C}_5\text{)} \rightarrow \text{PGA (C}_3\text{)} \rightarrow \text{PGA (C}_3\text{)}\]

Figure 2. Important steps in CO₂ fixation during C₃ photosynthesis. Some of these indicate the reverse steps through the reverse cycle (excluding the reverse steps according to the three nuclei available). Some of oxaloacetate, add ribulose concentration at 0,15, or various stages.
(ii) The C₄ (Hatch-Slack) pathway

- Somewhat less common for vascular plants
  - Exceptions: sugar cane, corn, bamboo (typical of plants in hot arid climates)
- Tropical grasses, desert plants, salt marsh plants use C₄ pathway.
- The adaptation allows maximum CO₂ fixation per unit loss of water.

**Characteristics:**
- optimum growth temp.: 35°C
- CO₂ compensation point: 0.0004% (1/100th of today’s atmosphere)
- light saturation: 10,000 ft cd
- max. photosynthetic rate: fast
- enzyme: phosphoenyl pyruvate (PEP) carboxylase
  - N.B. no competing oxygenase activity
  $\Delta^{13}$CO₂: -0.5 to -3.6 ‰ (ave. -2.2 ‰).

Plants using the C₄ pathways utilize PEP carboxylase for the first committed step in CO₂ fixation. The CO₂ fixed by PEP is carried as part of a C₄ acid from the mesophyll into the internal bundle sheath cells, where CO₂ is released again. The bundle sheath approximates a closed system, so most of CO₂ entering cell is fixed by RUBISCO to organic matter (minimal leakage) and internal CO₂ concentrations can be very high (100x atm.). Thus little isotopic fractionation is expressed in this step.

The smaller (ca. 2 ‰) isotope effect associate with the PEP carboxylase enzyme give C₄ plants more positive $\delta^{13}$C values (-8 to -18 ‰).
Environmental Conditions favoring C₄ photosynthesis

(a) Modeled net photosynthesis versus temperature for C₃ and C₄ plants (solid and dashed lines, respectively) in light-limited conditions. PAR incident on the leaf is 250 mmol m⁻² s⁻¹. The crossover temperature is the point at which the rates intersect.

(b) Modeled net photosynthesis versus temperature for C₃ and C₄ plants (solid and dashed lines, respectively) in light-saturated conditions. Impacts of stress that occur at temperature extremes are not included.

Still et al., 2003
The C4 fraction of the vegetation. Values below 0.005 are screened out.

Still et al., 2003

Carbon isotopic fractionation during C₄ photosynthesis

Model describing the isotopic fractionation in C₄ plants:

\[ \Delta = a + (b_4 + b_3 \phi - a) \times c/c_a \]

where:

- \( a \) is the isotope effect associated with diffusion of \( \text{CO}_2 \) into the plant
- \( b_4 \) is the isotopic effect with \( \text{CO}_2 \) diffusion in bundle sheath cells,
- \( b_3 \) is the fractionation associated with carboxylation (by PEP enzyme)
- \( \phi \) is the leakiness of the plant to \( \text{CO}_2 \)
- \( c/c_a \) is the concentration ratio of \( \text{CO}_2 \) internal to \( \text{CO}_2 \) external.

N.B. It has been shown that some algae (diatoms) can use a “C₄-like” pathway, i.e., possess \( \text{CO}_2 \) concentrating mechanisms (Reinfelder et al., 2000, Nature 407, 996-999).
Isotopic ranges (δ¹³C, permil) for terrestrial plant biomass and plant wax *n*-alkanes

<table>
<thead>
<tr>
<th></th>
<th>C3 plants</th>
<th>C4 plants</th>
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</thead>
<tbody>
<tr>
<td><strong>Bulk tissue</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>-20 to -35</td>
<td>-10 to -16</td>
</tr>
<tr>
<td>Average</td>
<td>-26</td>
<td>-13</td>
</tr>
<tr>
<td>Δbulk-alkanes</td>
<td>-7.7</td>
<td>-9.9</td>
</tr>
<tr>
<td><strong>n-Alkanes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>-34</td>
<td>-23</td>
</tr>
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Freeman & Colarusso, 2001

C3 and C4 annual gross primary production (GPP) by latitude. C4 GPP is the solid line; C3 GPP is the dashed line. Units are petagrams of carbon per year.

Still et al., 2003

Assimilation-weighted carbon isotope discrimination by latitude in per mil (%).
(iii) The CAM (crussulacean acid metabolism) pathway

- Used by succulents – cacti, crassulaceae
- Similar to C₄ pathway, but different spatial and temporal packing.
- Plants using this pathway have intermediate isotopic compositions between C₃ and C₄.
- Isotopically speaking, marine algae also generally fall between C₃ and C₄.
- CAM plants open their stomata at night (when temp and water loss is lowest) and produce malate (via PEP).
- The malate is then processed further during the day
- There is higher diffusive loss of CO₂ from CAM plants

(iv) Anoxygenic bacterial photosynthesis

- Doesn’t produce O₂; H₂ taken from H₂S instead of H₂O:
  \[
  \text{CO}_2 + 2\text{H}_2\text{S} \rightarrow \text{CH}_2\text{O} + \text{S}_2 + \text{H}_2\text{O}
  \]
- Some bacteria (e.g. purple photosynthetic bacteria) use RuDP pathway to fix CO₂ whereas others use an inversion of the Acetyl-CoA decarboxylation reaction - “reverse TCA cycle” (green photosynthetic bacteria).
- The latter is important in Black Sea (where the photic zone penetrates below the chemocline) - green sulfur bacteria (Chlorobiaceae) - anaerobic photoautotrophs.
  \[
  \text{Acetyl-CoA} + \text{CO}_2 \xrightarrow{\text{feridoxin}} \text{pyruvate} + \text{CoA}
  \]
- Fractionation factors range from -10 to -30 ‰
Isotopic fractionation in aquatic photoautotrophs

- Very complex, and not fully understood. This is because they may use more than one carbon fixation path, and more than one source of inorganic carbon.
- In general as [CO₂]ₐq decreases (due to high algal densities, elevated temps, fall in [CO₂]ₐm or increased pH) a shift toward heavier algal carbon is observed.
- Isotopic fractionation in aquatic plants is more complex. Because CO₂ diffuses more slowly in water than air, diffusion is often the limiting step.
- Many aquatic plants have some membrane-bound mechanism that actively transports dissolved inorganic carbon (DIC) into the photosynthesizing cells.
- If DIC (CO₂ and HCO₃⁻) concentrations are low, plants can “pump” DIC into cell.
- Plants grown at high DIC conc (5%) exhibit similar δ¹³C values to C₃ vascular plants.
- Plants grown at low DIC conc (0.03%) exhibit only a 5‰ fractionation.
- Model describing the isotopic fractionation in aquatic plants:
  \[ \Delta = d + b \times \left( \phi_o / \phi_i \right) \]
  - \(d\) is the equilibrium isotope effect between CO₂ and HCO₃⁻
  - \(b\) is the isotopic fractionation associated with carboxylation (by RUBISCO)
  - \(\phi_o / \phi_i\) is the ratio of CO₂ leaking out of the cell to the amount inside the cell.

Rubisco fractionation fully expressed
No enzymatic fractionation
Fractionation large when fixation controlled by RUBISCO kinetics.
Fractionation small when fixation controlled by mass transport
Algal $\delta^{13}C$ as a paleobarometer for atmospheric pCO$_2$?

General observations:
- $\delta^{13}C$ of suspended POC varies as a function of latitude (water temperature)
- $[CO_2(aq)]$ varies as a function of temperature
- Correlation between $\delta^{13}C_{POC}$ and $[CO_2(aq)]$.
- Relationship between $[CO_2(aq)]$ and pCO$_2$(atm)
- Past climate-related variations in pCO$_2$ (Vostok ice core).

Potential errors:
- Diffusive vs. non-diffusive (active) transport of CO$_2$
- Calibration of $\varepsilon_p$ versus $[CO_2(aq)]$
- Vertical position of algae in water column.
- Variations in photosynthetic mechanisms (C3 vs C4 pathways etc.)
- Other biological controls on fractionation
  - Growth rate (see Laws et al. 1995)
  - Cell size/shape (surface area)
  - Nutrient availability

Mixed layer $\delta^{13}C_{POC}$ plotted against (a) latitude and (b) temperature

Goericke & Fry 1994
Figure 2. (a) Mixed layer SST and (b) dissolved carbon dioxide [CO$_2$$_{eq}$] plotted against latitude and (c) mixed layer [CO$_2$$_{eq}$] plotted against SST. The lines in Figure 2c represent [CO$_2$$_{eq}$] as predicted (Widdows, 1974) for a mixed layer in equilibrium with an atmospheric pCO$_2$ of 325 ppmv (solid line) and 355 ppmv (dashed line). Seasonal variations of [CO$_2$$_{eq}$] at two stations off Iceland (64°N 20°W, solid line polygons) and in the Greenland Sea (68°N 10°W, dashed line polygons) are taken from Peng et al. (1987).

Goericke & Fry 1994

Figure 3. Values of $\varepsilon$, calculated from $^{81}$C$_{\text{PDB}}$ and $^{81}$C$_{(\text{CO}_2)_\text{aq}}$ plotted against [CO$_2$$_{eq}$] as predicted by Henry’s Law from SST (solid line in Figure 2b) for samples collected south (solid circles) and north (open circles) of the equator. Lines show linear regressions to all data (light line) and to data from $<$17 µmol [CO$_2$$_{eq}$] (heavy dashed line).

Goericke & Fry 1994
Relationship between carbon isotopic fractionation, [CO₂]aq & growth rate

\[ y = -0.025x + 0.771, \quad R^2 = 0.97 (n = 5) \]

**Fig. 2.** Relationship between \( \delta^{13} \text{C} \) and \( \mu \) for P. tricornutum grown in a chemostat/cycle system under light-dark cycles of 24h:18h and 12h:12h. The geometric mean-model III regression equation corresponds to the 24h:18h cycle, where \( \delta^{13} \text{C} \) ranged from 13 to 36 \%/oo. The open circle is the mean of the range of reported growth rates (0.080 d⁻¹) in the equatorial Pacific multiplied by 2.35 to correct for 1.5 cycles and respiration effects (see text) and divided by the (CO₂)aq of 10.8 μmol kg⁻¹. The corresponding \( \mu \) is 0.276. The error bars indicate the range of reported growth rates (0.47 - 7.94 d⁻¹). (Garric et al., 1994) and the variability associated with \( \mu \) (±1 standard deviation. Table 1). For the laboratory data, the standard error of the \( \mu \) measurements is ±5%. The standard error of the \( \delta^{13} \text{C} \) measurements is about ±3% of the mean value at the given growth rate.

Laws et al., 1995

**DIC concentrating mechanisms in algae**

Flows of carbon in an organism that actively assimilates inorganic carbon from its environment. For purposes of illustration it has been been assumed that the "pumped" form of carbon is bicarbonate, but there are also organisms in which CO₂ is the actively transported form.
Relationship between isotopic fractionation and phytoplankton cell geometry

Fig. 1. Schematic diagrams of Synechococcus sp., Emiliania huxleyi, Phaeodactylum tricornutum, and P. glacialis. Average cell dimensions and standard deviations are given as well as calculated average surface area to volume ratios. Note that P. glacialis is illustrated at 1/5 scale.

Popp et al., 1998
Summary of pathways of photosynthetic carbon fixation

In spite of differences in pathways by which carbon is supplied to biosynthetic reaction networks, sources of metabolic intermediates are similar for each system whereby carbohydrates are synthesized by the Calvin Cycle.

Organisms in the lower 2 groups, however, fix carbon at multiple reaction sites, resulting in large isotopic contrasts (both intermolecular and intramolecular).

Isotopic compositions of primary producers and other marine organisms
Influence of heterotrophic activity on isotope composition

• In general, the following assumption can be made (Hayes et al., 1990):
  – For multicarbon substrates, chemical reactions will not have a large effect on the molecular-
    average isotope compositions.
• A grazing organism that ingests particles does not discriminate on the basis of
  isotope composition. Consequently the isotopic composition of a given particle type
  should be no different from the starting material(s).
• The isotopic composition of a heterotroph can vary from that of its carbon source.
  – e.g. for respiratory processes:

\[
\text{CH}_2\text{O} + \text{oxidant (O}_2) \rightarrow \text{CO}_2 + \text{C in biomass (new tissue)} + \text{reduction product (H}_2\text{O)}
\]

• The isotopic difference between biomass and respired carbon depends on
  fractionation during metabolism.

\[
\delta_i = (1-f_b)\delta_d + f_b \times \delta_b
\]

where:
• \(i\) = input carbon
• \(d\) = respired \(\text{CO}_2\)
• \(b\) = biomass
• \(f_b\) = fraction of input carbon converted to biomass ("conversion efficiency")

Before a carbon atom can be lost a carbon-carbon bond must be broken (in most cases).
• \(^{13}\text{C}\) is lost more readily than \(^{12}\text{C}\) (i.e., respired \(\text{CO}_2\) is \(^{13}\text{C}\)-depleted).
• Therefore carbon retained as biomass is enriched in \(^{13}\text{C}\) relative to that respired.
• The isotope difference is typically 1 to 1.5 \(\%\), for organisms with low conversion
  efficiencies (\(f_b = 0.5 - 0.6\)).
• Water-dwelling invertebrates and protozoans have high conversion efficiencies
  \(\text{Average isotopic shifts per trophic level are expected to be less than 1.5 \%}\).
• Fermentative bacteria use biochemical processes that are markedly different from
  those in respiring heterotrophs. In general, the isotopic characteristics of these
  processes are poorly known, but have the potential for significant fractionations.
Stable isotopes as indicators of carbon flow in ecosystems

- Since there is generally considered to be minimal fractionation associated with aerobic heterotrophy, the potential exists to use isotopes as dietary indicators within food webs.
- "You are what you eat, plus 1 ‰"
- Isotope values "integrate" the diet
- A number of plant sources can be distinguished

Potential problems:
- Individual variability in δ¹³C averages 1 to 2 ‰ (masks assimilation effect)
- Results are often tissue or biochemical dependent.

But - a molecule passed on with an intact carbon skeleton should retain its original carbon isotopic composition*.
- *Important for molecular isotopic biogeochemistry since "surviving" molecules are frequently what we study
Stable Carbon Isotopes as Source Indicators

- Isotope composition of original plant material is almost unaffected by diagenetic alteration
- Most of the detritus in sediments are plant remains (phytoplankton, vascular plants)
- Much effort has been devoted to distinguishing contributions from these two sources based on $\delta^{13}$C values.
- Problems: most problems associated with accurately assigning end member compositions
  - End-members are unknown
  - End-members although known are temporally variable
  - There are more than 2 end-members

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<tr>
<td>POCE</td>
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<td>$\delta^{13}C$</td>
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<th>Isotope mixing models</th>
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<tr>
<td>$\delta^{13}C$</td>
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<td>Sample</td>
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A. 2 sources (50:50)
B. 2 sources (30:70), but $\delta$ value of one source modified by metabolic processes.
C. 3 potential sources

Carbon Isotopic differences between biochemicals

The immediate product of photosynthesis is glucose. However the metabolic conversion of glucose to other biochemicals often involves isotopic fractionations.

$$
\delta_{\text{biomass}} = f_{\text{NA}} \cdot \delta_{\text{NA}} + f_{\text{Prot}} \cdot \delta_{\text{Prot}} + f_{\text{PS}} \cdot \delta_{\text{PS}} + f_{\text{Lipid}} \cdot \delta_{\text{Lipid}}
$$

- where $f$ = mole fraction as C

- In general: $1%o < 5%o$
  $$
  \delta_{\text{PS}} > \delta_{\text{NA}} = \delta_{\text{Prot}} > \delta_{\text{Lipid}}
  $$
  Increasingly $^{13}C$ depleted

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<th>Isotopic variations between higher plant biochemicals</th>
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<tr>
<td>$\delta^{13}C$</td>
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Isotopic variations in vascular plant leaf biochemicals

Fig. 2. Average δ¹³C (% vs PDB) values for total tissue (T), total surface lipid extracts (S) and weighted mean n-alkanes (C₃₉) for each of the three plant groups (C₄, CAM and C₃). Error bars represent the standard deviation of the measurements for each fraction for the given plant group.

Depletion of ¹³C in lipids relative to marine algal biomass as a function of cellular composition

Components sum to yield biomass (Xₐ = mole fraction):

\[ X_{\text{Total}} + X_{\text{Prot}} + X_{\text{Sacc}} + X_{\text{Spr}} = 1 \; ; \; X_{\text{Prot}}/X_{\text{Sacc}} = 8.6 \]

Isotopic mass balance:

\[ X_{\text{C₃₉}} \delta_{\text{Prot}} + X_{\text{C₃₉}} \delta_{\text{Prot}} + X_{\text{C₃₉}} \delta_{\text{Sacc}} + X_{\text{C₃₉}} \delta_{\text{Spr}} = \delta_{\text{Biomass}} \]

\[ \delta_{\text{Prot}} \approx \delta_{\text{Sacc}}, \; \delta_{\text{Prot}} - \delta_{\text{Sacc}} \approx -1 \% , \; \delta_{\text{Spr}} - \delta_{\text{Sacc}} = -6 \% \]
Differences in $\delta^{13}C$ between individual biochemicals

"Compound-Specific Isotope Analysis" (CSIA)

Pioneering work by Abelson and Hoering (1961) on amino acids.

- Isotopic differences among individual amino acids in algae and bacteria have been observed (Macko et al., 1987).
- Isotopic differences among different lipid and pigment molecules have now been reported (Hayes et al., 1987; Freeman et al., 1990; Schouten et al., 1998).
- Isotopic relationships dictated by biosynthetic pathways (starting substrates and number of branching points in pathway).

- For lipids:
  - Acetogenic lipids (based on acetate units)
  - Isoprenoid lipids
    - Mevalonoid pathway
    - Methylerythritol phosphate pathway (only recently recognized).

Carbon Isotopic distributions within molecules

- Glucose is considered to be isotopically homogeneous.
- Lipids exhibit sawtooth $\delta^{13}C$ distributions down linear carbon chains with the carboxyl-derived carbons from the acetyl-CoA being about 6‰ lighter than the methyl-derived carbons.
- Monson and Hayes (1982) demonstrated alternating isotope pattern and related it to:
  - (a) overall depletion of $^{13}C$ in fatty acid lipid fraction and
  - (b) the biochemical pathway of fatty acid formation.
Pathways of lipid biosynthesis in eukaryotic organisms

Complexity in isotopic compositions is introduced by presence of subcellular organelles. Since acetate cannot pass through mitochondrial wall, C₂ units produced there by decarboxylation of pyruvate are packaged as C₆ species (citrate) for export. Formation and subsequent conversion of C₆ to C₂ units provides possibilities for isotopic fractionation during biosynthesis of lipids.
Pathways of lipid biosynthesis in prokaryotic organisms

Pathways of lipid biosynthesis in prokaryotic organisms. In heterotrophs, any of the indicated multi-carbon organic compounds might derive from the food source. In Calvin-Cycle autotrophs the effective internal source of organic carbon is a C6 carbohydrate. For organisms using the reverse-TCA or acetyl-CoA systems of carbon fixation, the methyl and carboxyl positions of acetyl-CoA will derive from two different fixation sites. The letters m and c denote positions in the C5 and C2 biomonomers that are derived from the methyl and carboxy positions in acetyl-CoA and indicate the different m/c ratios in the two lipid families.

Pathways of isoprenoid synthesis: Isotopic implications

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*aka Deoxyxylulose pathway
Synthesis of cyclic isoprenoids (triterpenoids)

Isotopic distribution within isoprenoids
Factors bearing on isotopic fractionation in aquatic photoautotrophs

<table>
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<tr>
<th>Organisms</th>
<th>Isoprenoid biosynthesis</th>
<th>Oxidative pentose phosphate pathway in plastid?</th>
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<tr>
<td></td>
<td>$C_{20}, C_{40}$</td>
<td>$C_{15}, C_{20}$</td>
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<tr>
<td>‘Normal’</td>
<td>MEP</td>
<td>MVA</td>
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<td>Green Algae</td>
<td>MEP</td>
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<td>Euglenoids (flagellates)</td>
<td>MVA</td>
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<tr>
<td>Cyanobacteria</td>
<td>MEP</td>
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</table>

$C_{20}$ and $C_{40}$ generally plastidic (formed in chloroplast)
$C_{15}$ and $C_{20}$ commonly cytosolic
MEP = methylerythritol-phosphate pathway
MVA = mevalonic acid pathway

Depletion of $^{13}$C in isoprenoid lipids produced by eukaryotic algae

![Depletion graph](image-url)
Stable carbon isotopic composition in *T. minimum* (freshwater green alga)

Continuous cultures

Batch cultures