Carbon Isotopes and Isotopic Fractionation for Complex Processes

Reading:
Valley and Cole Chapters 3 (Hayes), 10 (Des Marais), and 11 (Freeman)
White, Lecture 28 Optional: Faure, 1986, Chapter 26

Motivation:
C, S, N, and other redox-active elements have mass-dependent fractionation that is largely controlled by multi-step, predominantly biological processes
- C by photosynthesis
- S by sulfate reduction
- N by nitrification, volatilization, denitrification
- Fe, Cr, Se, and Mo: various redox reactions, may be biologically mediated
We need a general framework for understanding how isotopic fractionation varies according to reaction rates and reaction pathways
Photosynthesis is the most important driver of C isotope fractionation, but there are other important isotopic effects we must understand in order to use C isotope data
Carbon isotopes provide important records for understanding earth history.

Guide Questions:
- In general, how does isotopic fractionation vary multi-step chemical reactions that are predominantly forward reactions (far from equilibrium)? How do reaction rates and reaction pathways influence the overall isotopic fractionation of the reaction product relative to the reactant?
- How does photosynthesis fit this model? What are the various steps involved and how do they differ between C$_3$ and C$_4$ plants? What are the resulting ranges of isotopic fractionation? How does CO$_2$ concentration affect the isotopic fractionation?
- What are the isotopic fractionations between the dissolved inorganic carbon species, roughly?
- Are the molecules that make up living tissues in plants and animals all the same in their C isotope composition? If not, how large are the $\delta^{13}$C differences between different classes of molecules (roughly)?
- What are the $\delta^{13}$C values of the atmosphere, C$_3$ plants, C$_4$ plants, marine organic matter, marine carbonate rock, and methane?
- The $\epsilon$ value for photosynthesis was apparently lower at times in the past. What does this suggest about conditions at that time?
- The $\delta^{13}$C value of marine carbonate has varied over time? What do larger/smaller $\delta^{13}$C values suggest about the marine carbon cycle?
- How can we constrain animal and human diets in the past using C and N isotopes?
- In studies of contaminant transport and biodegradation, what two types of information can be gained from C isotope data?
- Can C and H isotopes be used to determine how a methane deposit formed? If so, what is the general idea?
Isotopic fractionation by multi-step chemical processes (e.g., photosynthesis, sulfate reduction, abiotic redox processes)

Highly simplified model:

Each step could have some fractionation; what is the fractionation for the whole process?

Need to develop the concept of a rate-limiting step (from kinetic theory):
- Sequence of steps; each step has a forward rate constant $k$: Rate = $k \times$ conc.
- Each step also has a back reaction with its own rate constant
- Assume that one step has a much smaller forward rate constant than the others
- This is the rate-limiting step (RLS); the “bottleneck”
- Steps after the RLS cannot go any faster than the RLS-
  - limited by supply coming from the RLS
  - intermediates do not accumulate, no back reaction
  - “100% conversion” at each step- no isotopic fractionation
- Steps before the RLS: Each step proceeds forward until its products build up to the point where the back-reaction is as fast as the forward (= equilibrium)
- Thus: Steps before RLS are in equilibrium and later steps are kinetic

How does this affect isotopic fractionation of the overall process?

**Extreme Case 1:** Diffusion into the cell is rate-limiting:
There is a kinetic effect (small) at the RLS related to diffusion differences.
Later steps: All the reactants are passed through (100%)- no net fractionation
Overall fractionation = that of the diffusion step alone.

**Important general principle:** If 100% of the reactant is consumed by the next step (i.e., “passed through”), there can be no isotopic fractionation.

**Extreme Case 2:** Diffusion is NOT rate limiting. RLS is the bond-breaking step (large $\varepsilon$).
1) In this case, diffusion relatively fast, and the concentration inside the cell ~ that outside.
2) Thus, there is an outward flux of reactant, and the isotopic fractionation of the band-breaking step thus influences the reactant pool outside the cell.
3) This happens when cells are metabolizing very slowly (e.g., they are starved of electron donors or maybe have little light for photosynthesis)

More generally, let’s look at a 3 step process where each step can fractionate isotopes, and the last step is the rate limiting step.
1) Steps One and Two: Products of these steps build up, because the third step is the slowest. 2) The concentrations build up to the point where back reaction = forward, i.e., equilibrium occurs. 3) The products of the steps (the various pools) are thus fractionated as follows:

\[ \delta_0 \hspace{1cm} \delta_0 + \Delta_1 \hspace{1cm} \delta_0 + \Delta_1 + \Delta_2 \hspace{1cm} \delta_0 + \Delta_1 + \Delta_2 + \varepsilon_3 \]

**General principles for isotopic fractionation in a multi-step kinetic process:**

1) Total fractionation = sum of equilibrium fract’s for all steps prior to the rate-limiting step PLUS the kinetic fractionation at the rate limiting step. 2) Steps after the rate limiting step have no effect on the overall isotope fractionation 3) If there are two steps in the chain that are rate limiting, i.e., there are two slow steps with about the same rate constant, then the isotopic fractionation has an intermediate value.

Note: If this seems self-contradictory, e.g., “You told us there’s an isotope effect for all these steps, but then you told us the effects of some steps don’t contribute to the overall fractionation for the process- how can this be?”…..

- The key is in the delta values of the small inventories/pools of intermediate products
- These are driven to isotopically heavy values because lighter isotopes are preferentially consumed by the reactions
- The smaller the concentration of intermediate, the greater the tendency for this to happen
- Concentration of intermediates are low if they are consumed by fast reactions
Isotopic Fractionation Induced by Photosynthesis:
This covered in John Hayes’ chapter in Valley and Cole (Ch. 3).

Note on the definition of $\varepsilon$: There are three different definitions of $\varepsilon$ in the literature:
1. Hayes seems to use: $\varepsilon = -(\delta_{\text{product}} - \delta_{\text{reactant}})$ convenient because $\varepsilon$ is $>0$ for kinetic processes
2. Various papers: $\varepsilon = \delta_{\text{product}} - \delta_{\text{reactant}}$ the same, but $\varepsilon$ is negative
3. Canfield: $\varepsilon = 1000(\alpha - 1)$ where $\alpha = R_{\text{reactant}}/R_{\text{product}}$ – in this case, epsilon is again positive for a kinetic process.
   - Definition 3 is the most rigorous, and I use this one.
   - Definition 1 is a close approximation to definition 3 when the delta values are close to 0‰.
   - Definition 2 is used in a few papers, and is the simplest, but is less rigorous.
   - The bottom line: It is convenient to speak about kinetic isotope effects using per mil values- alpha is too messy (e.g., a 0.2 per mil fractionation means $\alpha = 1.0002$). Whichever definition you choose, state it clearly in your writings and be careful and consistent about – vs. + signs.

1) $C_3$ Plants: Calvin cycle: Algae, autotrophic bacteria, most land plants
   …vs. $C_4$ plants: sugar cane, Maize/corn, hot-region grasses
   …Also there are CAM plants (both mechanisms)

See Fogel and Cifuentes, 1993 Fig.1 for sketch of RuBP reaction
-and their Fig. 3 for C isotope fractionation by plants as a function of CO$_2$ conc.

Picture an algal cell, with CO$_2$ diffusing in, and some CO$_2$ inside diffusing out.

\begin{center}
\includegraphics[width=\textwidth]{diagram.png}
\end{center}

\begin{itemize}
  \item CO$_2$ (out)
  \item CO$_2$ (in)
\end{itemize}

- Step 1: Diffusion; Kinetic, $\varepsilon < 4.4\%$
- Step 2: Carboxylation via RuBP (Enzyme); Kinetic, $\varepsilon = 29.4\%$

Later steps (no effect)
What is the resulting overall fractionation?

Case 1: If step 2 is rate-limiting: Add fractionation for steps 1 and 2. The fractionation for step one is probably small- reversible diffusion (unless there’s some conversion to HCO$_3^-$ in cell fluids).

When does this occur? When CO$_2$ is so plentiful that CO$_2$ concentration inside the cell is affected little by photosynthetic uptake, and thus, the CO$_2$ concentration inside the cell is nearly equal to that outside.

Case 2: If step 1 is rate-limiting: Only the fractionation at step 1 affects the overall fractionation.

When does this occur? When CO$_2$ is not plentiful, and CO$_2$ concentration inside the cell is drawn down by photosynthetic uptake, and thus, the CO$_2$ diffuses mostly inward with very little escape of unreacted CO$_2$ out of the cell.

Case 3: In most cases, diffusion DOES limit the rate SOMEWHAT. This case is intermediate between cases 1 and 2 and the fractionation is also intermediate.

The extent to which diffusion is rate-limiting depends on the CO$_2$ concentration inside, relative to outside. So the fractionation is expresses as a function of $C_{in}/C_{out}$.

The bottom line on this:

$\varepsilon = a + (c_i/c_o)(b-a)$

$a =$ step 1 fract.

$b =$ step 2 fract.

$c_i =$ CO$_2$ conc. inside

$c_o =$ CO$_2$ conc. outside

(White’s uses $\Delta$ instead of $\varepsilon$)

**Theoretical line:** $\varepsilon = 4.4 + 23(C_{in}/C_{out})$ (seems like real plants have somewhat smaller fractionations than predicted by theory, but the general trend is correct and values are similar)

Generally observed $\varepsilon$ values for C$_3$ plants: 16‰ to 26‰

- Depends on all variables affecting plant’s rate of photosynthesis
- Faster rates mean lower conc. inside

Generally observed $\delta^{13}C$ values for C$_3$ plants: 23‰ to 33‰ (atmospheric CO$_2$ $\delta^{13}C = 7‰$)

**IMPORTANT:** we expect that isotopic fractionation decreases when CO$_2$ conc. in the atmosphere decreases. Thus, if we can find some way to determine the $\varepsilon$ for photosynthesis at some time in the past and compare it to today’s value we may have an indication of past CO$_2$ concentrations.
2) **C₄ Plants**: sugar cane, Maize/corn, hot-region grasses; these plants were later to evolve than C₃ plants, and proliferated in mid-latitude areas relatively recently

The equation for C₄ plants is (according to White)
\[ \varepsilon = a + (b_{234} + b_5\phi - a) \left( \frac{c}{c_a} \right) \]
Use \( \varepsilon \) instead of \( \Delta \) like White
\n- \( a = \text{step 1 fract.} \)
- \( b_{234} = \text{steps (2+3+4) fract.} \)
- \( b_5 = \text{step 5 fract.} \)
- \( \phi = \text{fraction of CO}_2 \text{ leaked to outside from the inner sheath cells’} \)

The equation for C₄ plants is (according to Hayes; better reference)
Note: Hayes uses a definition of \( \varepsilon \) such that \( \varepsilon \) is positive for a kinetic process. Hence, my version below is slightly different than his, with a sign change on \( b_2 \).
\[ \varepsilon = a + [b_2 + b_5 + \phi(b_5 - b_{\text{leak}}) - a] \left(1 - f\right) \]
\( a = \text{step 1 fract.} \) (diffusion, atm into plant; \( \varepsilon = 4.4\% \))
b₂ = step 2 fract. (equilibrium between CO₂ and bicarbonate, both inside; Δ =+8‰)
b₃ = step 3 fract. (PEP binding of bicarb, ε = 2.2‰)
b₅ = step 5 fract. (RuBP reaction, ε = 25‰)
b_leak = fract. occurring with leakage of CO₂ out of sheath cells
φ = fraction of CO₂ leaked to outside from the inner sheath cells (varies between plants, but
f is the fraction of carbon taken into the plant that actually makes it through the reaction
Note that (1-f) can be related to CO₂ concentrations inside the plant and outside the plant as
before (inside meaning inside the plant but not inside the sheath cells yet)
ε = a +[b₂+b₃ + φ(b₅-b_leak) -a ](c_i/c_a)

The bottom line:
Average ε for C₄ plants is about 4‰
δ¹³C for C₄ plants is -11‰ (atm CO₂ = -7‰ )
• (versus C₃ plants = -20 to -30‰)
C₄ plants are also less sensitive to changes in CO₂ conc. in the atmosphere.
This all makes sense in a general way:
• C₄ plants have extra steps in their carbon intake system that shuttle CO₂ into inner
areas of the plants that are separated from the outside world. This makes them
less leaky, the carboxylation step that causes the big 25‰ fractionation is not
much in contact with the outside environment, and thus the overall fractionation
for photosynthesis is smaller than for C₄ plants.

Data from real C₃ plants
Data from real C₄ plants

φ =0.34 line
φ =0.21 line

3) CAM plants (Cacti) can use either C₃ or C₄ pathways- intermediate results

4) Marine algae:
a. Use HCO₃⁻ instead of CO₂, usually
Less fractionation (relative to CO₂ supply) than land plants, because the CO₂ -
HCO₃⁻ equilibrium involves an equilibrium fractionation- the HCO₃⁻ is heavier by
7 to 12 ‰
b. Diffusion is slower in water than in air
…so fractionation strongly dependent on the CO$_2$ (actually, DIC = dissolved inorganic carbon = total of CO$_2$ + HCO$_3$$^-$ + CO$_3^{2-}$) concentration. Marine algae have pump mechanisms that actively transport DIC into cell, thus cell conc. is higher than outside.

$\varepsilon = d + b_3(F_3/F_1)$ (Note: White has a typo in his version)

$d =$ step 1 fract. - equilibr. Between CO$_2$ + HCO$_3$$^-$ ($\Delta = +8.5 \%$) 
$b_3 =$ fract for carboxylation (-29\%) 
$F_3/F_1 =$ fraction of the CO$_2$ leaked out of the cell

**Bottom line:**
$\varepsilon$ for marine algae is variable
- can be as low as 5\% when DIC conc. is low,
- can be as high as for land plants (29\% enrichment in lighter isotopes) when DIC conc. is high (rare)
- ballpark common range: 10-15\%

5) **Fresh water algae:** Somewhat like marine algae, but often pH is lower (vs. ocean = 7.5 to 8.5), HCO$_3^-$ utilization less important

### Equilibrium fractionations for DIC species

<table>
<thead>
<tr>
<th>Species</th>
<th>$\Delta$ vs. CO$_2$(gas)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO$_2$ (dissolved)</td>
<td>-0.8</td>
</tr>
<tr>
<td>HCO$_3^-$</td>
<td>+7.7</td>
</tr>
<tr>
<td>CO$_3^{2-}$</td>
<td>+6.4</td>
</tr>
<tr>
<td>CaCO$_3$</td>
<td>+9.8</td>
</tr>
</tbody>
</table>

(calc’d using Faure’s parameters, room T)

### Biosynthesis- additional, smaller C isotope fractionation between different molecules that are manufactured (and between differently bonded carbons in a single molecule)

This process fractionates isotopes somewhat (<10\%), so different components of an organism will have different $\delta^{13}$C values.
- lipids tend to be lighter than other components by 2 to 8\%
  - general tendency to have lighter C in C-H bonded groups (lipids $\rightarrow$ petroleum)
  - general tendency to have heavier C in C-O bonded groups (sugars, cellulose, amino acids)
- makes some sense in terms of equilibrium theory (but these reactions are kinetic).
  - Hoefs fig. 50 for $\delta^{13}$C of components of plant material
Carbon isotopes on Earth

1. Ranges of various carbon reservoirs on earth: (δ¹³C measured versus PDB (or VPDB)-carbonate rock)
   - Mantle: -3 to -7 ‰
   - Atmosphere ~ -7‰
   - Terrestrial Plants ~ -8 to -35‰
   - Marine organic matter in seds. ~ -15 to -35‰
   - Seawater HCO₃⁻ = ~ -2‰
   - Marine Carbonate precipitated ~ 0‰
   - Methane ~ -10 to -80‰

   NOTE: atmospheric CO₂ δ¹³C has changed over time. For example, fossil fuel combustion has released C with a relatively low δ¹³C value into the atmosphere, causing a shift in δ¹³C (about 0.2‰ between 1982 and 1990, for example). Any disturbance in the global C cycles will tend to change the atmospheric δ¹³C value.

Some applications (there are MANY others):

1. If fractionation during photosynthesis by marine algae depends on CO₂ conc. in the water (and thus, in atmosphere), then we should see changes in δ¹³C as the CO₂ conc. changes from glacial to interglacial-
   - Kate Freeman’s chapter in the Valley and Cole Book describes this. Also see:
   - Example: In 1994 Jasper and Hayes used the following relationship between the C isotope fractionation during manufacture of organic matter and the dissolved CO₂ concentration: ε = -32.9 log(C) +14.3 (C is micromolar dissolved CO₂ in ocean)

   Example 2: Late Precambrian glaciations, around 750 Ma: Negative excursions in δ¹³C, along with decreases in ε. Smaller fractionation implies low CO₂ in atmosphere, and resulting cold climate have been the driver of the glaciations.

2. Over the last 4 billion years, major changes in the earth’s carbon cycle are reflected in carbon isotopes in ocean seds.
   - See Des Marais chapter in Valley and Cole
   - Organic C vs. carbonate burial
     - Organic C burial removes light C, drives ocean DIC to greater δ¹³C
ii. Carbonate burial removes heavy C, drives ocean DIC to lesser $\delta^{13}C$

iii. If organic C burial dominates, ocean DIC quite heavy ($> +20\%$)

iv. If carbonate burial dominates, ocean not so heavy

Currently, 20% of the carbon burial is organic C
(Des Marais, from Valley and Cole Book; Fig. 3)

- Before oxygenic photosynthesis:
  i. Organic carbon synthesis only about 20 Teramoles per year
  ii. CO$_2$ outgassing from mantle about the same size

- After oxygenic photosynthesis:
  i. Organic carbon synthesis about 9000 Teramoles/yr.
  ii. What happens if this is mostly buried?

- Changes on earth at about 2.2 Ga:
  i. Huge swings to greater $\delta^{13}C$
  ii. Presumably reflects greater burial of organic matter
  iii. Oxygenic photosynthesis cranked up production of organics
  iv. Mostly buried until scavenging organisms caught up

- Negative excursions in $\delta^{13}C$ of carbonate around 750 Ma- lower productivity during snowball earth conditions? Little organic burial.

3. Similarly: C isotope profiles across the KT boundary- carbonate rocks record a decrease in $\delta^{13}C$ just after the KT boundary
   - Probably reflects strong changes in global C cycle after a meteorite impact
   - We expect lower productivity, therefore, less burial of isotopically light C
     \[ \Rightarrow \text{This uptake normally drives DIC toward heavier values} \]
     \[ \Rightarrow \text{Less of this uptake means lighter values} \]

4. The diet of animals and hominids can be determined from the $\delta^{13}C$ (and $\delta^{15}N$) values. Food remains in pot shards are one possibility for something to measure, teeth and bones are possible for $\delta^{13}C$ (see White’s Chapter 34)
   - Legumes have distinctive N isotope ratios
   - Maize is a C$_4$ plant- shifts observed in anthropological sites
   - Diets of marine species can also be traced (Paul Koch, UC Santa Cruz and others)
   - Birds, too.
5. Also, $\delta^{13}C$ is influenced by biological productivity in water. $\delta^{13}C$ is a function of depth in a body of water where photosynthesis occurs in the top layer. Lighter isotopes removed preferentially, converted to solid particles. If productivity was less in the past (maybe because of climate change or nutrient flux change) we expect that the $\delta^{13}C$ will reflect this. Productivity of the oceans is critical to global carbon cycle issue- if productivity declines, less C is sucked out of the atmosphere.

6. Degradation of organic groundwater contaminants often involves a kinetic isotope effect. The remaining contaminant becomes progressively heavier with increasing degradation. This provides a means of determining degradation rates.

7. If there are multiple sources of pollution in one area, it may be possible to “fingerprint” the source of contaminants with $\delta^{13}C$ measurements (and $\deltaD$). $\delta^{37}Cl$ may also provide an indicative tracer for Cl-bearing compounds like TCE.

**Methane: Determining mechanism of production**

**3 pathways for creation of methane:**

1. **Fermentation:** Methane is generated by microbes breaking organic molecules into smaller ones; e.g., acetate cleaves into $\text{CH}_4 + \text{CO}_2$  
   Methane produced is very light isotopically.

2. **CO$_2$ reduction:** CO$_2$ is a terminal electron acceptor for bacteria- methane produced, and it is somewhat lighter than the starting CO$_2$. Usually in marine sediments.

3. **Thermogenic gas:** abiotic breakage of molecules, with different fractionation

These three types of gas can be distinguished by C and H isotopes:

![δD-δ13C diagram](image)