# Carbon isotopic fractionation during $C_3$ photosynthesis

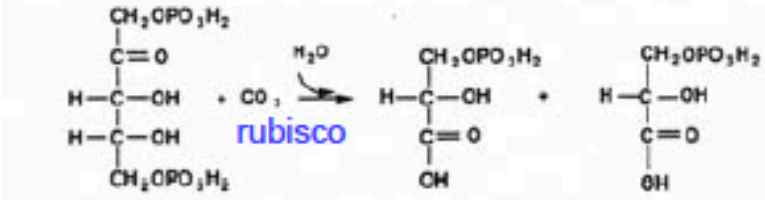
Model describing the isotopic fractionation, $\Delta$, in $C_3$ plants:

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

where

- $a$ is the isotope effect associated with diffusion of $CO_2$ into the plant (~ 0.8 to 4.0 ‰)
- $b$ is the fractionation associated with carboxylation (by RUBISCO enzyme)
- $c_i/c_a$ is the concentration ratio of $CO_2$ internal to $CO_2$ external.
- When $c_i/c_a = 1$ (i.e. unlimited $CO_2$) max RUBISCO fractionation, $b$ expressed.
- When $c_i/c_a << 1$ (i.e. limited $CO_2$) diffusion limited, and only $a$ expressed.

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




RuBP ($C_5$) PGA ($C_3$) PGA ($C_3$)

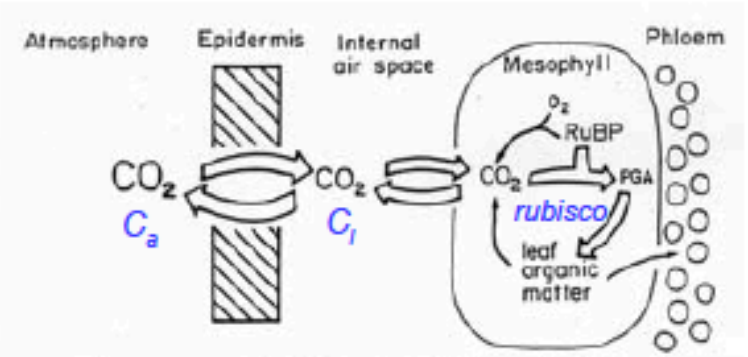


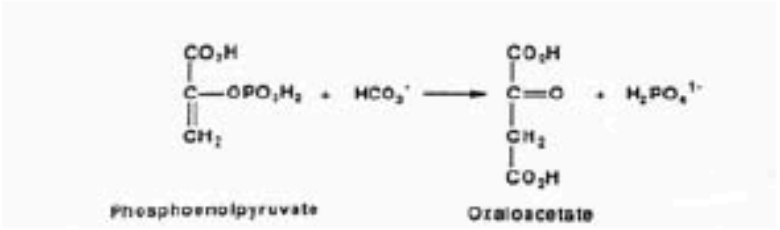

Figure 2. Important steps in $CO_2$ fixation during $C_3$ photosynthesis. Sizes of arrows indicate the relative fluxes through the various steps (including the reverse steps) according to the best models available. Sizes of symbols reflect relative concentrations of $CO_2$ at various stages.

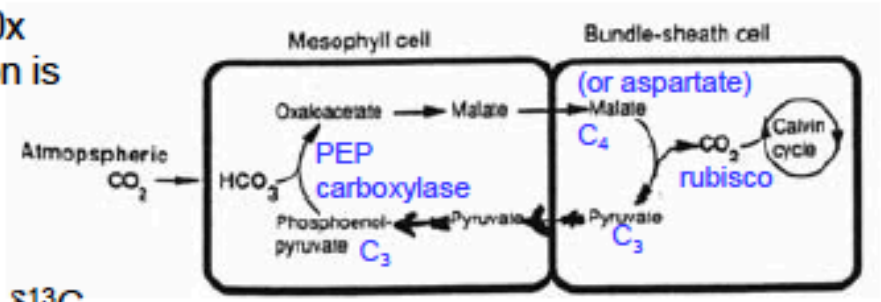
# (ii) The $C_4$ (Hatch-Slack) pathway

Plants using the $C_4$ pathways utilize PEP carboxylase for the first commited step in $CO_2$ fixation. The $CO_2$ fixed by PEP is carried as part of a $C_4$ acid from the mesophyll into the internal bundle sheath cells, where $CO_2$ is released again. The bundle sheath approximates a closed system, so most of $CO_2$ entering cell is fixed by RUBISCO to organic matter (minimal leakage) and internal $CO_2$ concentrations can be very high (100x atm.). Thus little isotopic fractionation is expressed in this step.

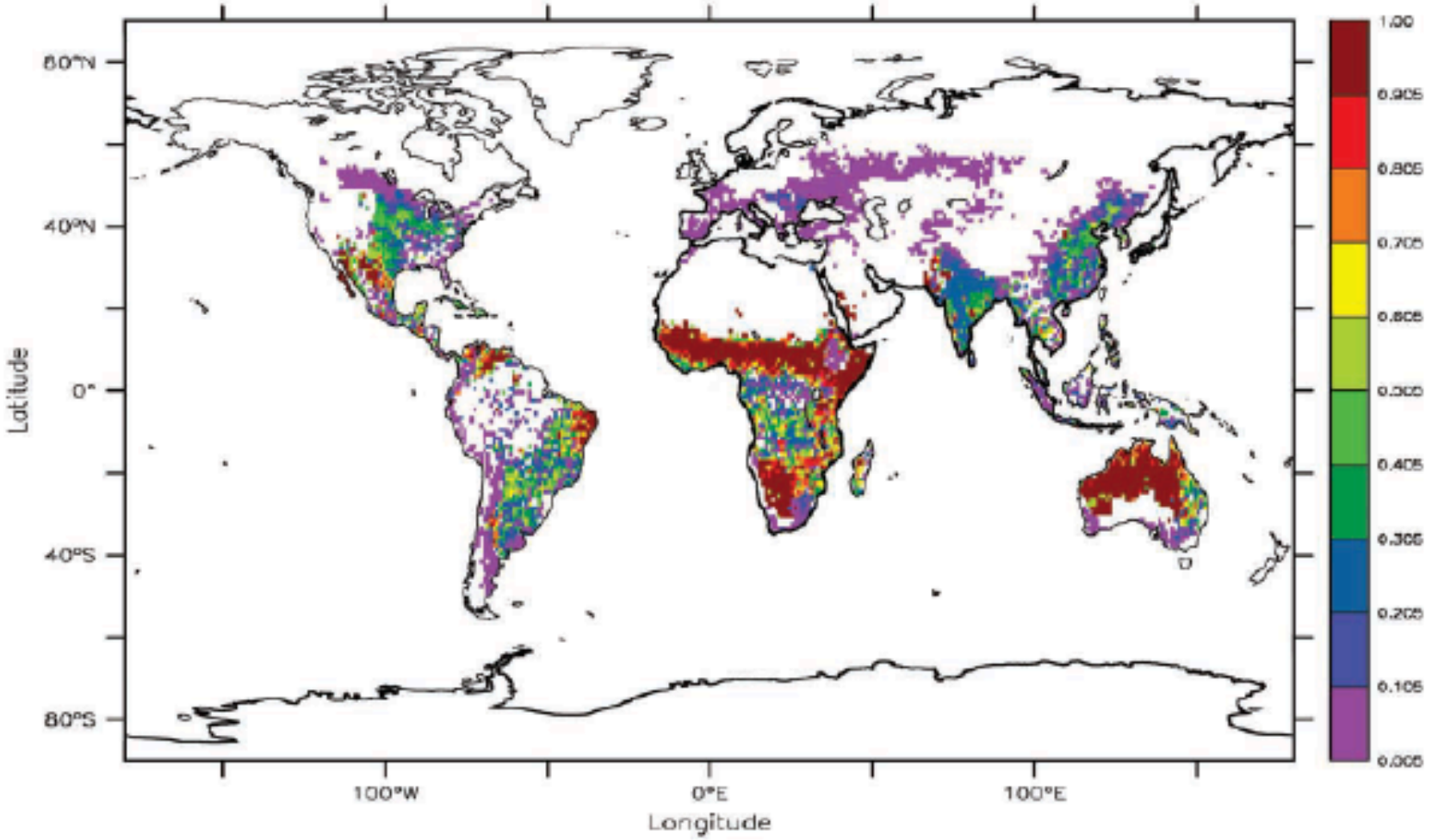
The smaller (ca. 2 ‰) isotope effect associate with the PEP carboxylase enzyme give $C_4$ plants more positive $\delta^{13}C$ values (-8 to -18 ‰).

Formation of oxaloacetate ($C_4$) from phosphenolpyruvate by the PEP carboxylase reaction




The C4 fraction of the vegetation. Values below 0.005 are screened out.




Still et al., 2003

# Carbon isotopic fractionation during $C_4$ photosynthesis

Model describing the isotopic fractionation in $C_4$ plants:

$$\Delta = a + (b_4 + b_3\,\phi - a) \times c_i/c_a$$

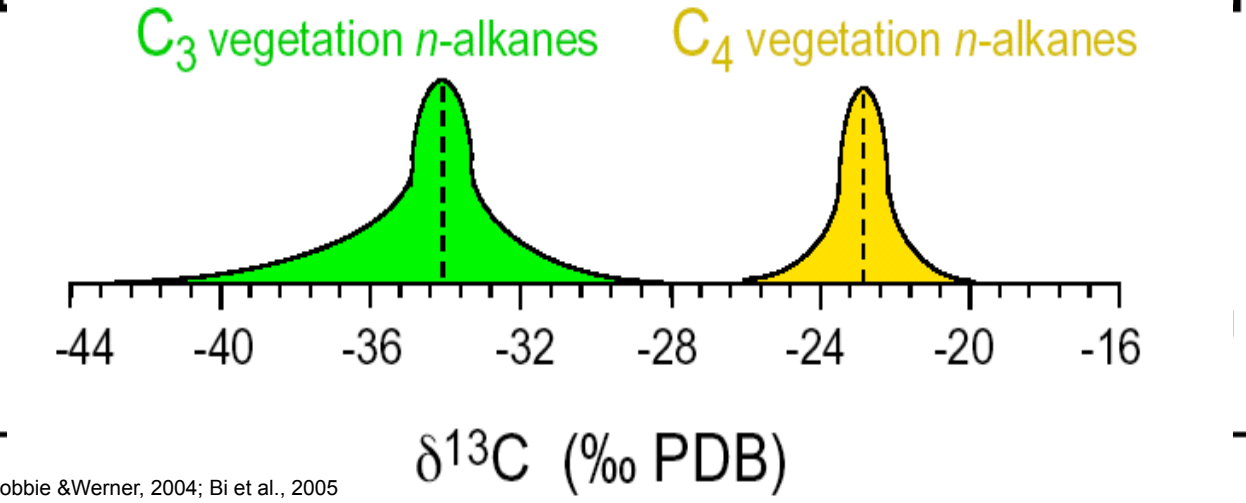
where:

- $a$ is the isotope effect associated with diffusion of $CO_2$ into the plant
- $b_4$ is the isotopic effect with $CO_2$ diffusion in bundle sheath cells,
- $b_3$ is the fractionation associated with carboxylation (by PEP enzyme)
- $\phi$ is the leakiness of the plant to $CO_2$
- $c_i/c_a$ is the concentration ratio of $CO_2$ internal to $CO_2$ external.

*N.B. It has been shown that some algae (diatoms) can use a "$C_4$-like" pathway, i.e., possess $CO_2$ concentrating mechanisms (Reinfelder et al., 2000, Nature 407, 996-999).*

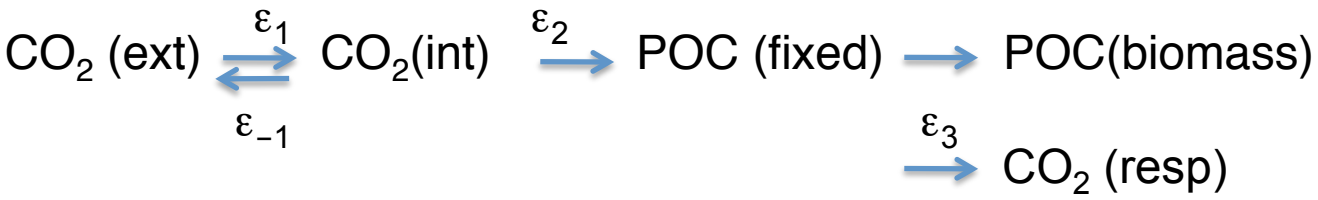
## Isotopic ranges ($\delta^{13}C$, permil) for terrestrial plant biomass and plant wax *n*-alkanes

| | C3 plants | C4 plants |
|---|---|---|
| **Bulk tissue** | | |
| Range | -20 to -35 | -10 to -16 |
| Average | -26 | -13 |
| Δbulk-alkanes | -7.7 | -9.9 |
| | | |
| **n-Alkanes** | -28 to -43 | -20 to -26 |
| Average | -34 | -23 |




Collister et al., 1994; Hobbie &Werner, 2004; Bi et al., 2005

## Is the isotopic value of POM related to atmospheric $CO_2$?




In this case the isotopic value of $CO_2$ (internal) is impacted by the diffusion of $CO_2$ back out of the cell

$$\delta_{CO2(int)} = \delta_{CO2(ext)} - \varepsilon_1 + f\,\varepsilon_{-1} + (1-f)\,\varepsilon_2$$

Where $f = k_{-1}/k_1$ or $[CO2]_{int}/[CO2]_{ext}$

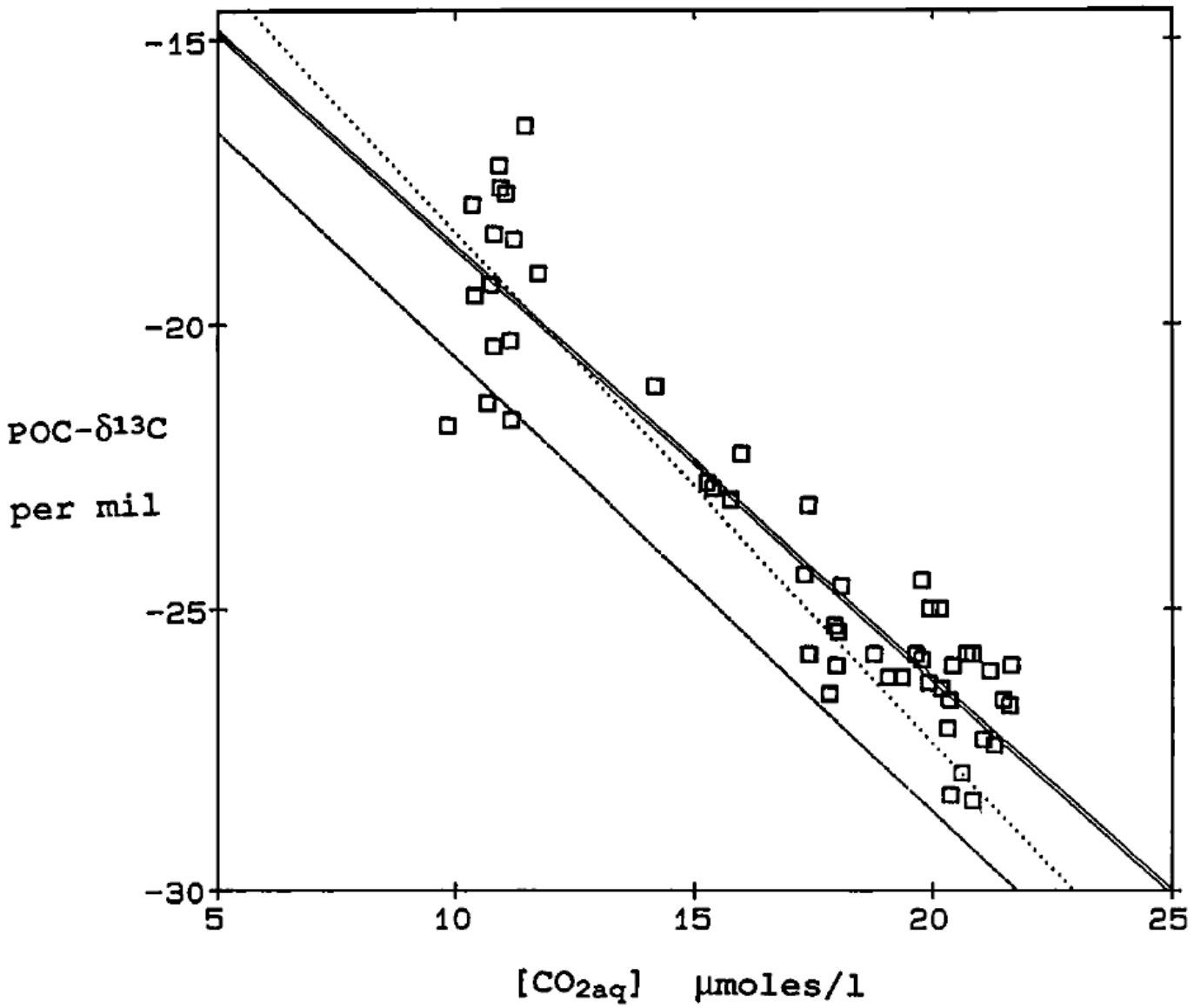
Substituting this expression back into the equation for the relationship between POC and CO2:

$$\delta_{POC} - \delta_{CO2(ext)} = -\varepsilon_1 + [CO2]_{int}/[CO2]_{ext}\,(\varepsilon_{-1} - \varepsilon_2)$$

or

$$[\delta_{POC} - \delta_{CO2(ext)} + \varepsilon_1]/(\varepsilon_{-1} - \varepsilon_2) = [CO2]_{int}/[CO2]_{ext}$$

Therefore, the isotopic difference between POC and $CO_2$ reflects changes in $[CO2]_{ext}$. suggesting that  can be used as a paleobarometer of $CO_2$.




Rau et al, 1992

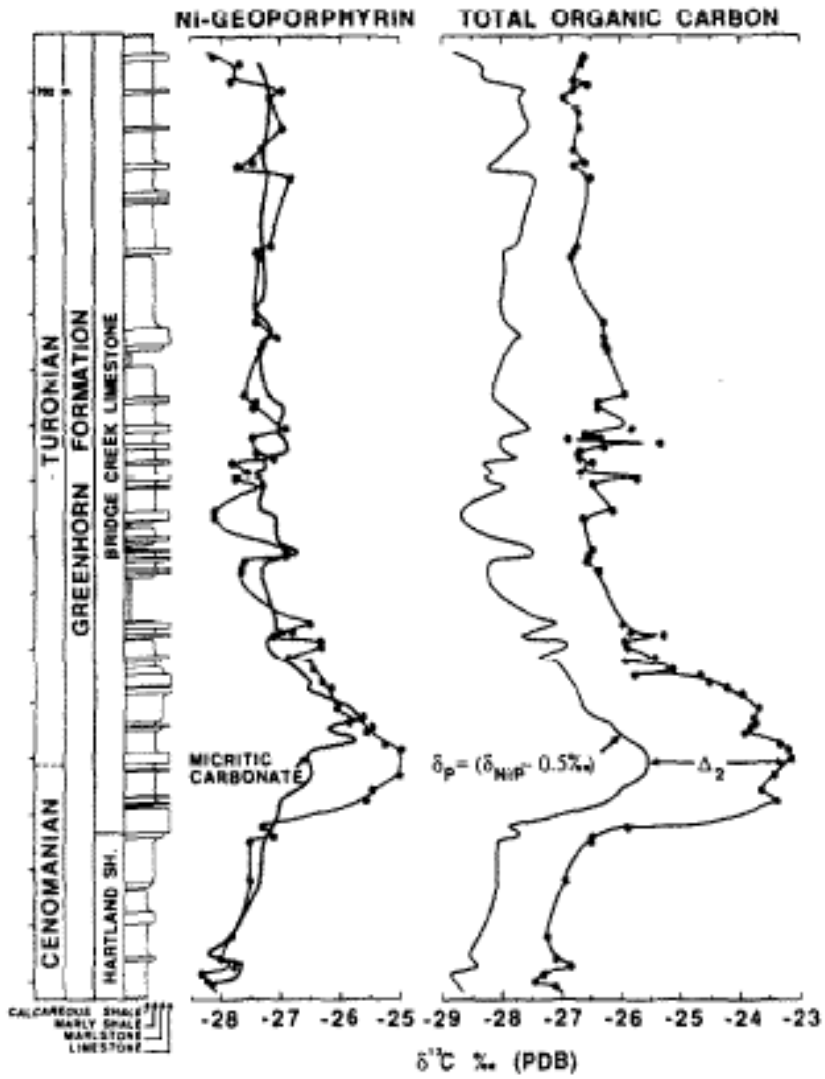



FIG. 3. Carbon isotopic compositions of Ni-geoporphyrins and total organic carbon plotted as a function of stratigraphic position.

**Is the isotopic value of POM related to growth rate?**

Substrate → Product

$\delta_{substrate} - \delta_{product} = \varepsilon$

*In algae, total carbon production is the sum of growth and respiration*:

$P = \mu + v$ where $\mu$ = specific growth rate, v = specific respiration.

The flow of carbon from $CO_2$ to can be expressed as:

$CO_2$ → POC(fixed) ($\varepsilon_1$) → $CO_2$(resp) ($\varepsilon_3$) → growth

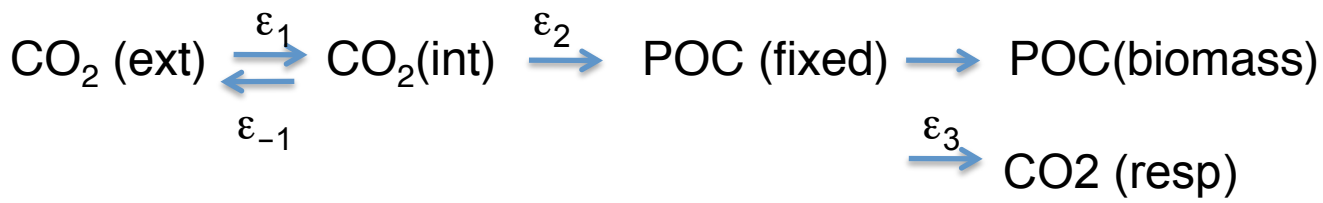
For isotopic mass balance:

$$\delta_{C(fixed)}(\mu+v) = \delta_{POC}\mu + (\delta_{POC} - \varepsilon_3)v$$

If $\delta_{CO2} - \varepsilon_1 = \delta_{C(fixed)}$ and rearranging for $\delta_{POC}$:

$$\delta_{POC} = \delta_{CO2} - \varepsilon_1 + \varepsilon_3(v/\mu+v)$$

For $C_3$ plants, the situation is a bit more complex because the cells are leaky




In this case the isotopic value of $CO_2$ (internal) is impacted by the diffusion of $CO_2$ back out of the cell

$$\delta_{CO2(int)} = \delta_{CO2(ext)} - \varepsilon_1 + f\,\varepsilon_{-1} + (1-f)\,\varepsilon_2$$

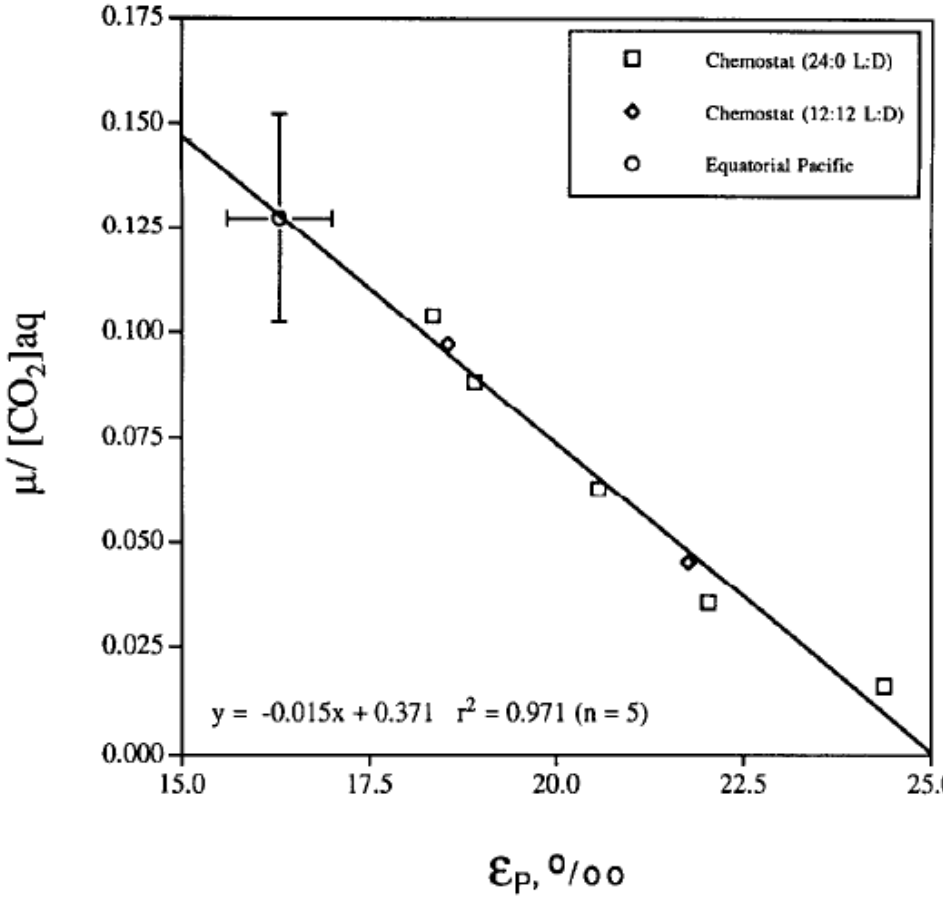
Where $f = k_{-1}/k_1$ or $[CO2]_{ext}/[CO2]_{int}$

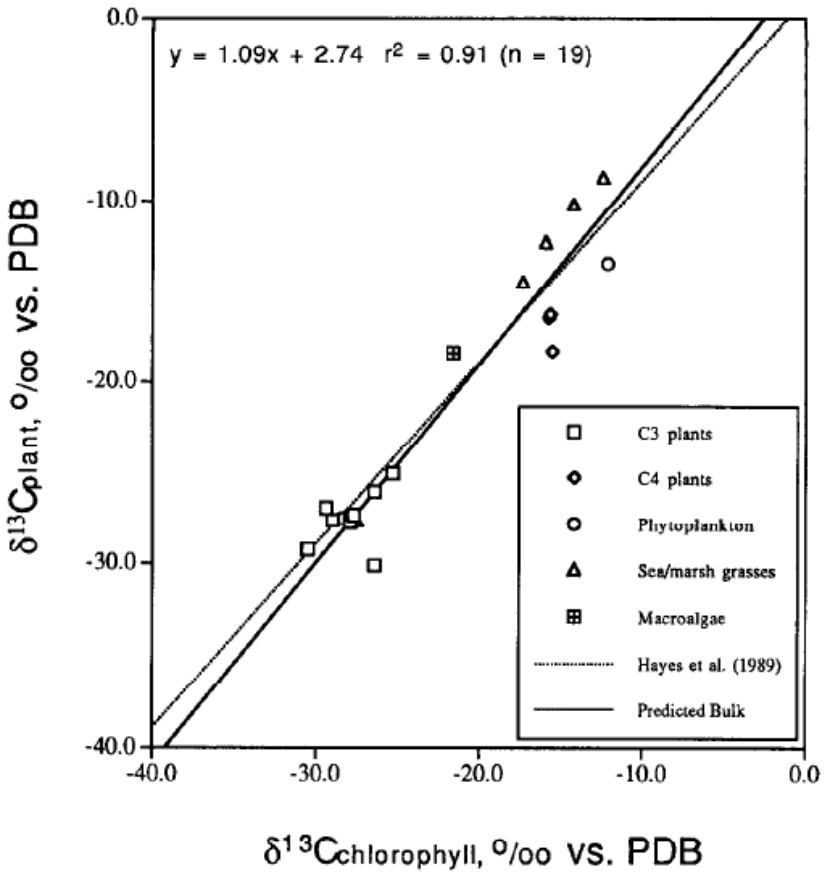
Substituting this expression back into the equation for the relationship between POC and CO2:

$$\delta_{POC} = \delta_{CO2(ext)} - \varepsilon_1 + f(\varepsilon_{-1} - \varepsilon_2) + \varepsilon_3(v/\mu+v)$$

We can relate the isotopic value of POC with external $CO_2$ through fractionations associated with diffusion into and out of the cell, fixation and respiration.

Carbon isotope composition of phytoplankton



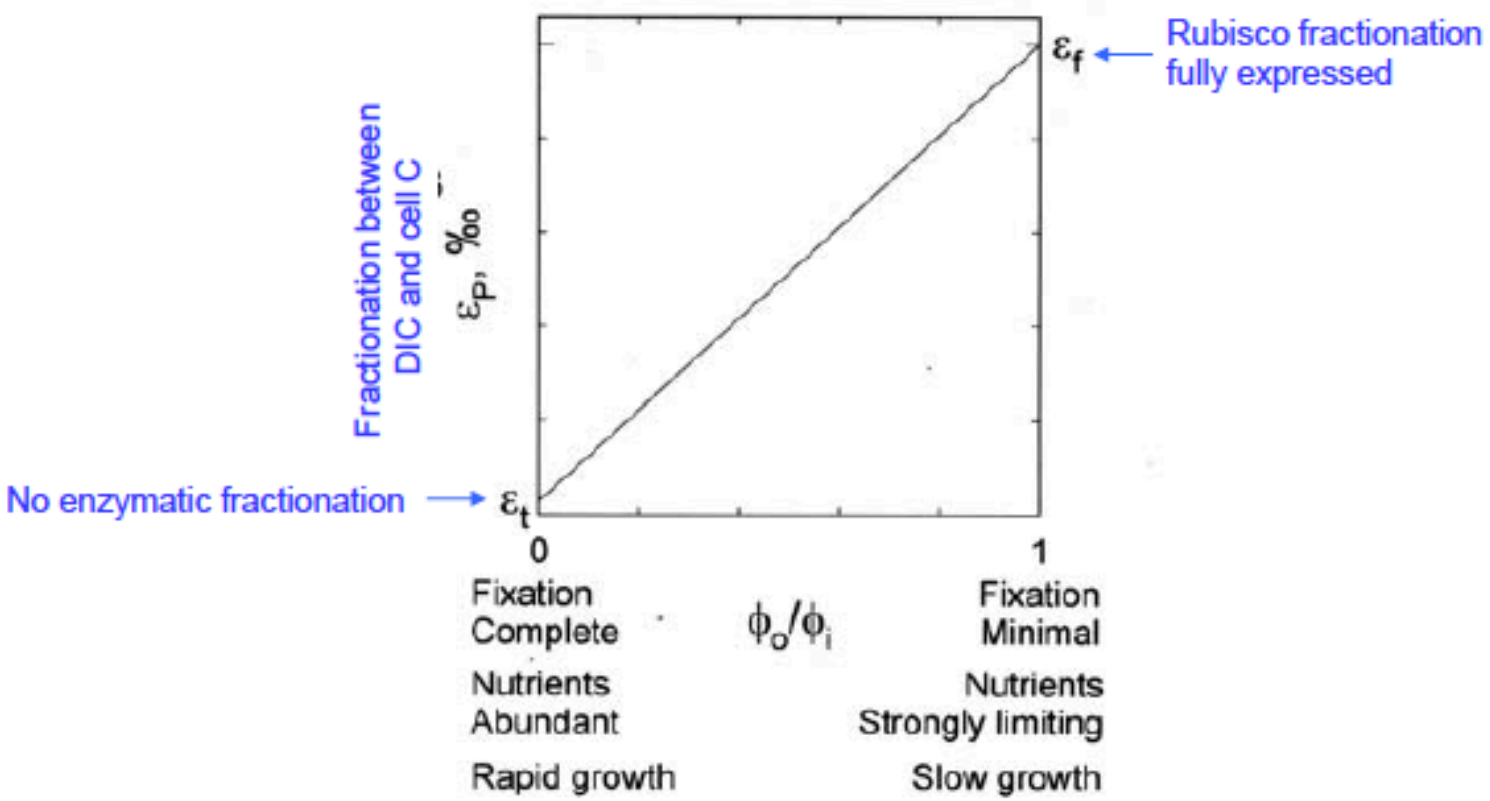
# Isotopic fractionation in aquatic photoautotrophs

- Very complex, and not fully understood. This is because they may use more than one carbon fixation path, may have carbon concentrating mechanisms, and more than one source of inorganic carbon.
- In general as $[CO_2]_{aq}$ decreases (due to high algal densities, elevated temps, fall in $[CO_2]_{atm.}$ or increased pH) a shift toward heavier algal carbon is observed.
- Isotopic fractionation in aquatic plants is more complex. Because $CO_2$ 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 ($CO_2$ and $HCO_3^-$) concentrations are low, plants can "pump" DIC into cell.
- Plants grown at high DIC conc$^n$ (5%) exhibit similar $\delta^{13}C$ values to $C_3$ vascular plants.
- Plants grown at low DIC conc$^n$ (0.03%) exhibit only a 5 ‰ fractionation.
- Model describing the isotopic fractionation in aquatic plants:

$$\Delta = d + b \times (\phi_o / \phi_i)$$

- Where:
  - $d$ is the equilibrium isotope effect between $CO_2$ and $HCO_3^-$
  - $b$ is the isotopic fractionation associated with carboxylation (by RUBISCO)
  - $\phi_o / \phi_i$ is the ratio of $CO_2$ leaking out of the cell to the amount inside the cell.

Typically, $\varepsilon_1 < \varepsilon_2$




Fractionation *large* when fixation controlled by RUBISCO kinetics.
Fractionation *small* when fixation controlled by mass transport

# 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:

$$CH_2O + \text{oxidant } (O_2) \rightarrow CO_2 + \text{C in biomass (new tissue)} + \text{reduction product } (H_2O)$$

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

# Influence of heterotrophic activity on isotope composition

The isotopic fractionation during metabolism can be summarized as:

$$\delta_i = (1\text{-}f_b)\delta_d + f_b \times \delta_b$$

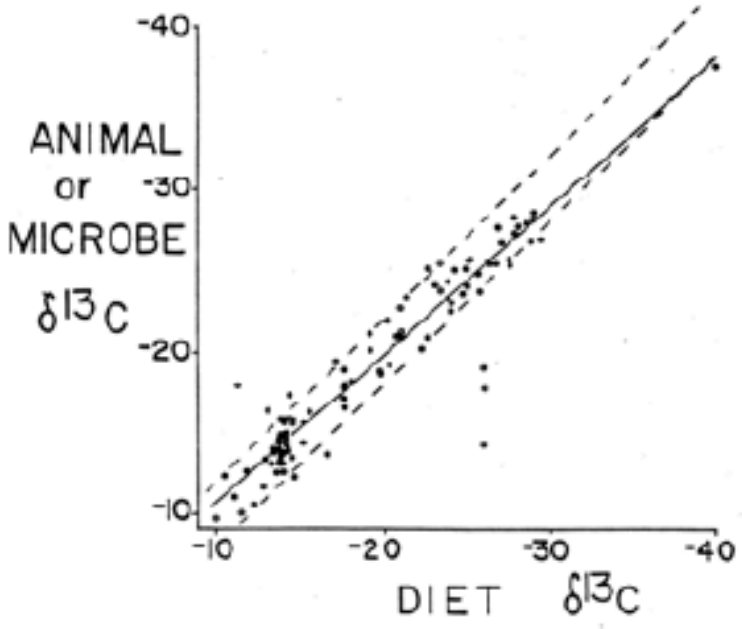
where:

- i = input carbon
- d = respired $CO_2$
- b = biomass
- $f_b$ = fraction of input carbon converted to biomass ("conversion efficiency")

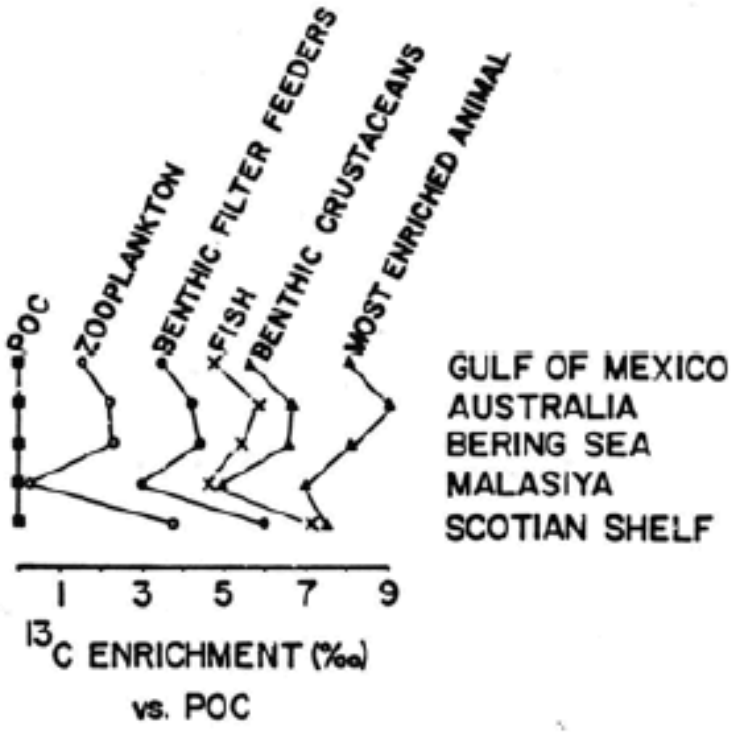
Before a carbon atom can be lost a carbon-carbon bond must be broken (in most cases).

- $^{12}C$ is lost more readily than $^{13}C$ (i.e., respired $CO_2$ is $^{13}C$-depleted).
- Therefore carbon retained as biomass is enriched in $^{13}C$ 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
- *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.

Isotope relationships between animals (and microbes) and diet




$^{13}C$ enrichment in marine ecosystems



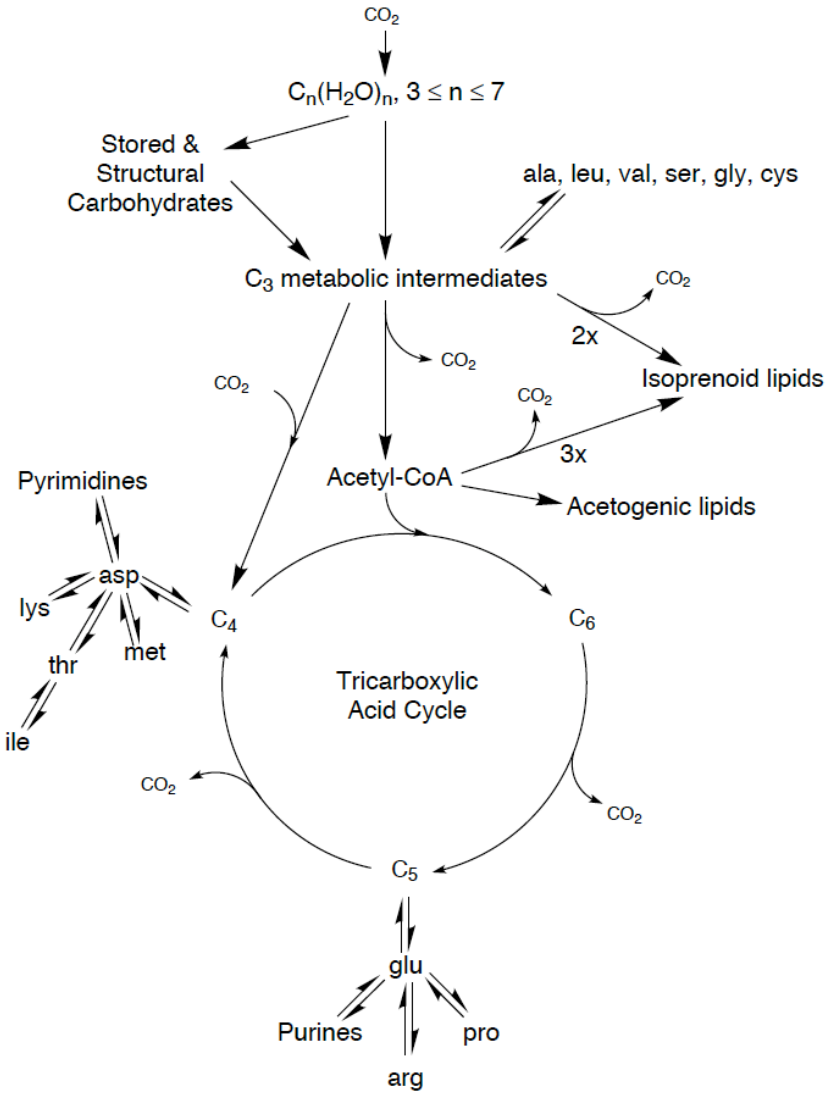
# Stable isotopes as indicators of carbon flow in ecosystems

- 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 $\delta^{13}C$ 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*

# Isotopic fractionation within different functional classes of organic matter



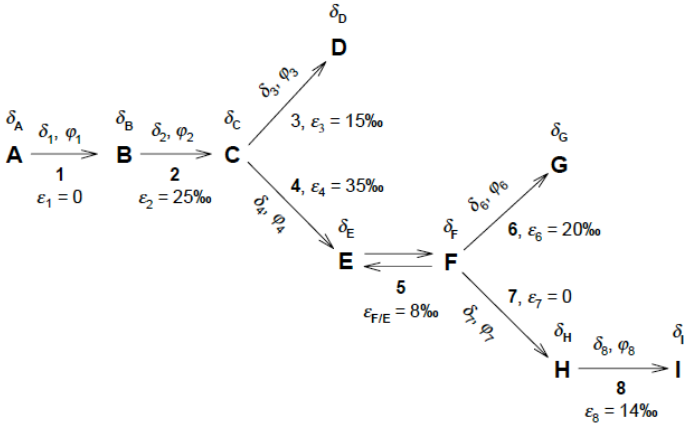




**Figure 2.** A network of chemical reactions. Letters indicate carbon positions within reactants and products. Isotopic compositions of these positions are indicated by $\delta$s with alphabetical subscripts. Reactions are designated by numbers and the $\delta$s, $\varphi$s, and $\varepsilon$s with numerical subscripts indicate respectively isotopic compositions of the carbon being transmitted by a reaction, the flux of carbon being transmitted (moles/time), and the isotope effect associated with the reaction. The latter value is expressed in ‰ and $\varepsilon$ is defined in the text accompanying equation 1.

# 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.

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

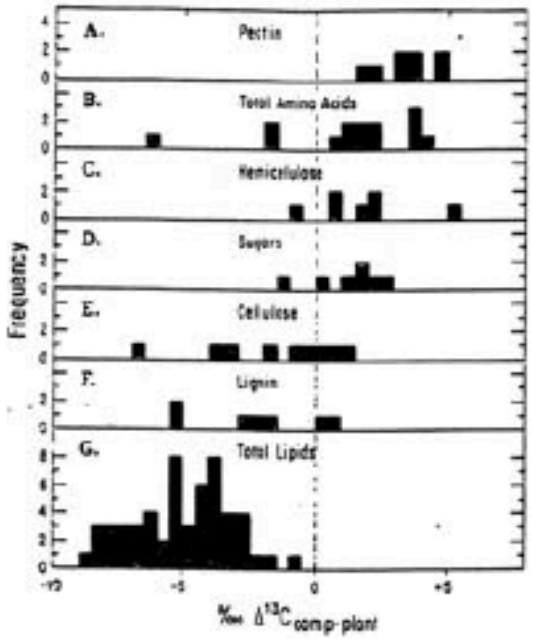
– where $f$ = mole fraction as C

- In general: 1‰ 5‰

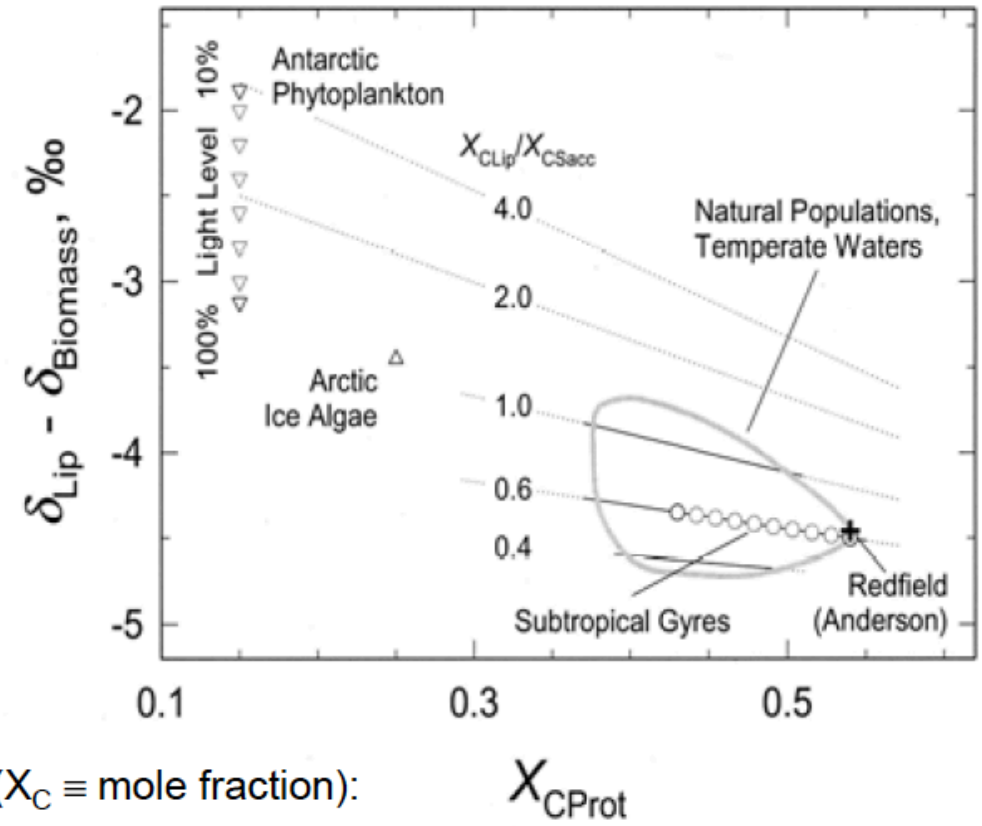
$$\delta_{PS} > \delta_{NA} \approx \delta_{Prot} > \delta_{Lipid}$$

*Increasingly $^{13}C$ depleted*

Isotopic variations between higher plant biochemicals



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




Components sum to yield biomass ($X_C \equiv$ mole fraction):

$X_{Cna} + X_{Cprot} + X_{Csacc} + XC_{lip} = 1$; $X_{Cprot}/X_{Cna} = 8.6$

Isotopic mass balance:

$X_{Cna}\delta_{na} + X_{Cprot}\delta_{prot} + X_{Csacc}\delta_{sacc} + X_{Clip}\delta_{lip} = \delta_{biomass}$

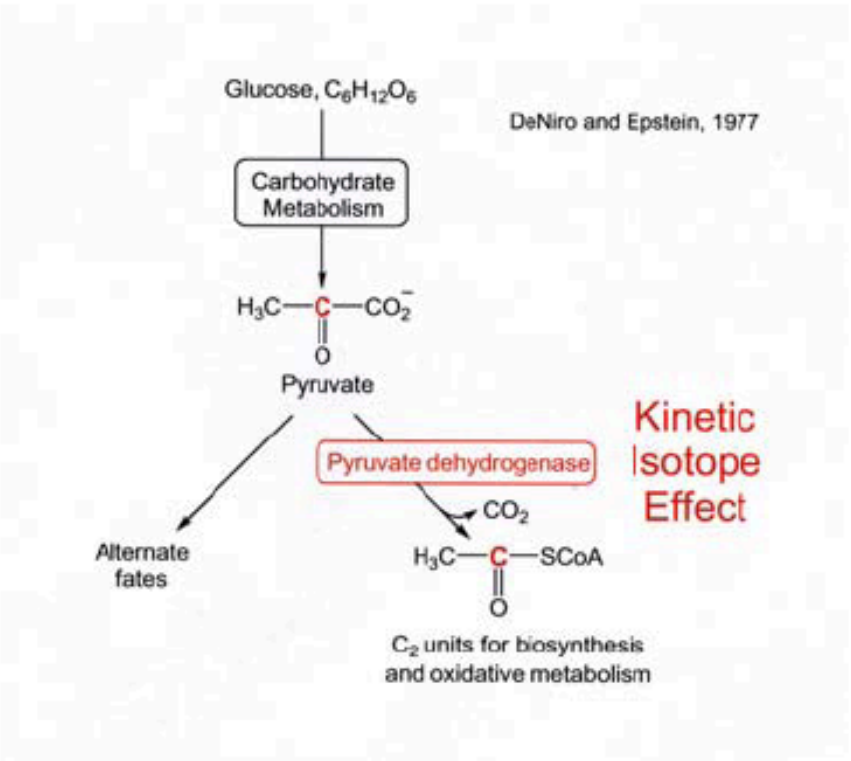
$\delta_{na} \approx \delta_{prot}$, $\delta_{prot} - \delta_{sacc} \approx -1$‰, $\delta_{lip} - \delta_{sacc} = -6$ ‰

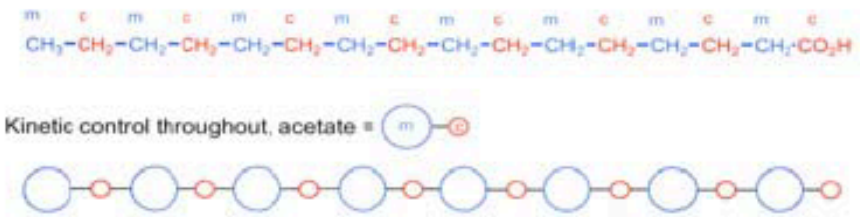
# Carbon Isotopic distributions within molecules

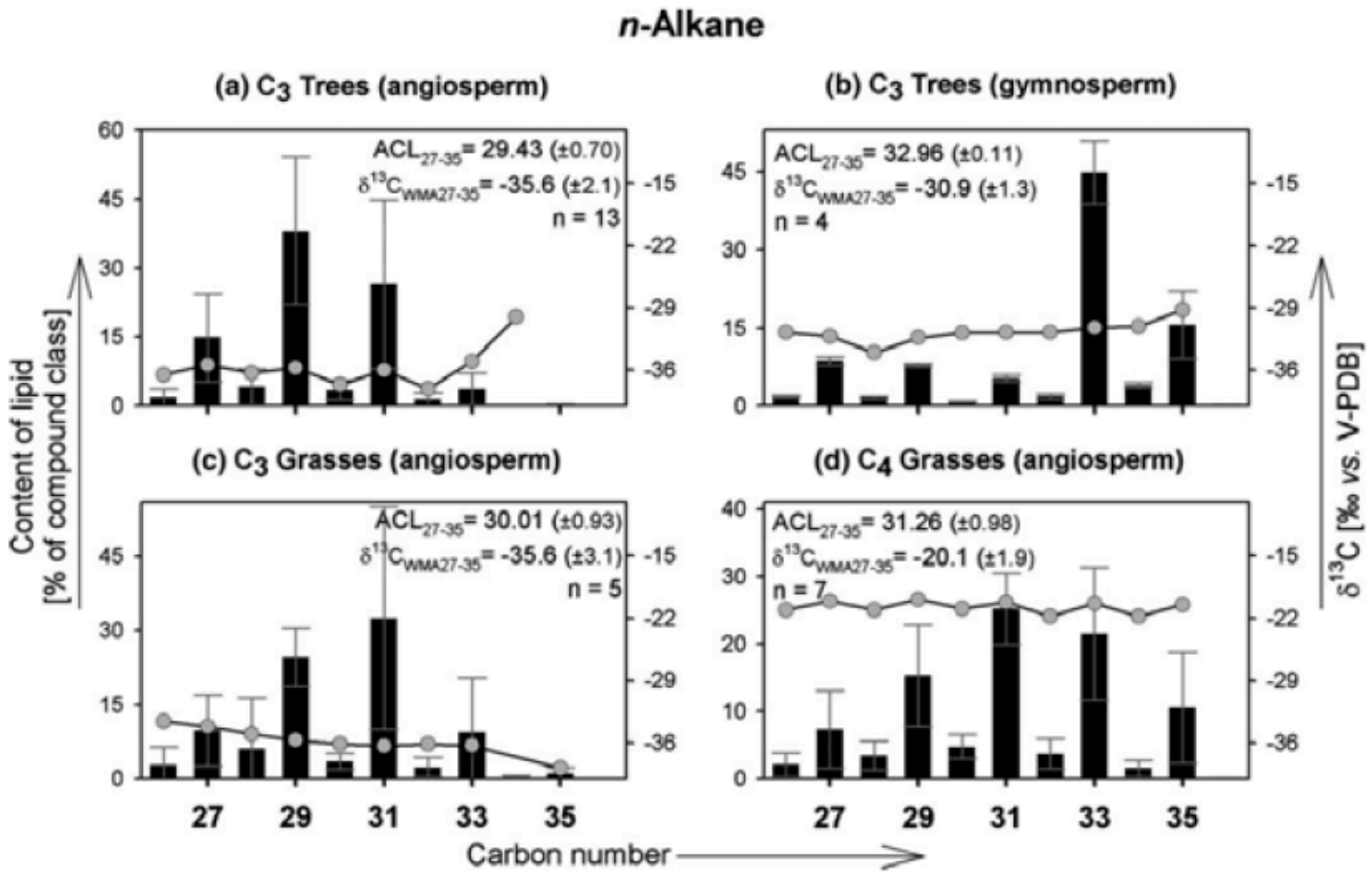
- Glucose is considered to be isotopically homogeneous
- Lipids exhibit sawtooth $\delta^{13}C$ distributions down linear carbon chains with the carboxyl-derived carbons from the acetyl-coA being about 6 ‰ lighter than the methyl-derived carbons.
- Monson and Hayes (1982) demonstrated alternating isotope 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.



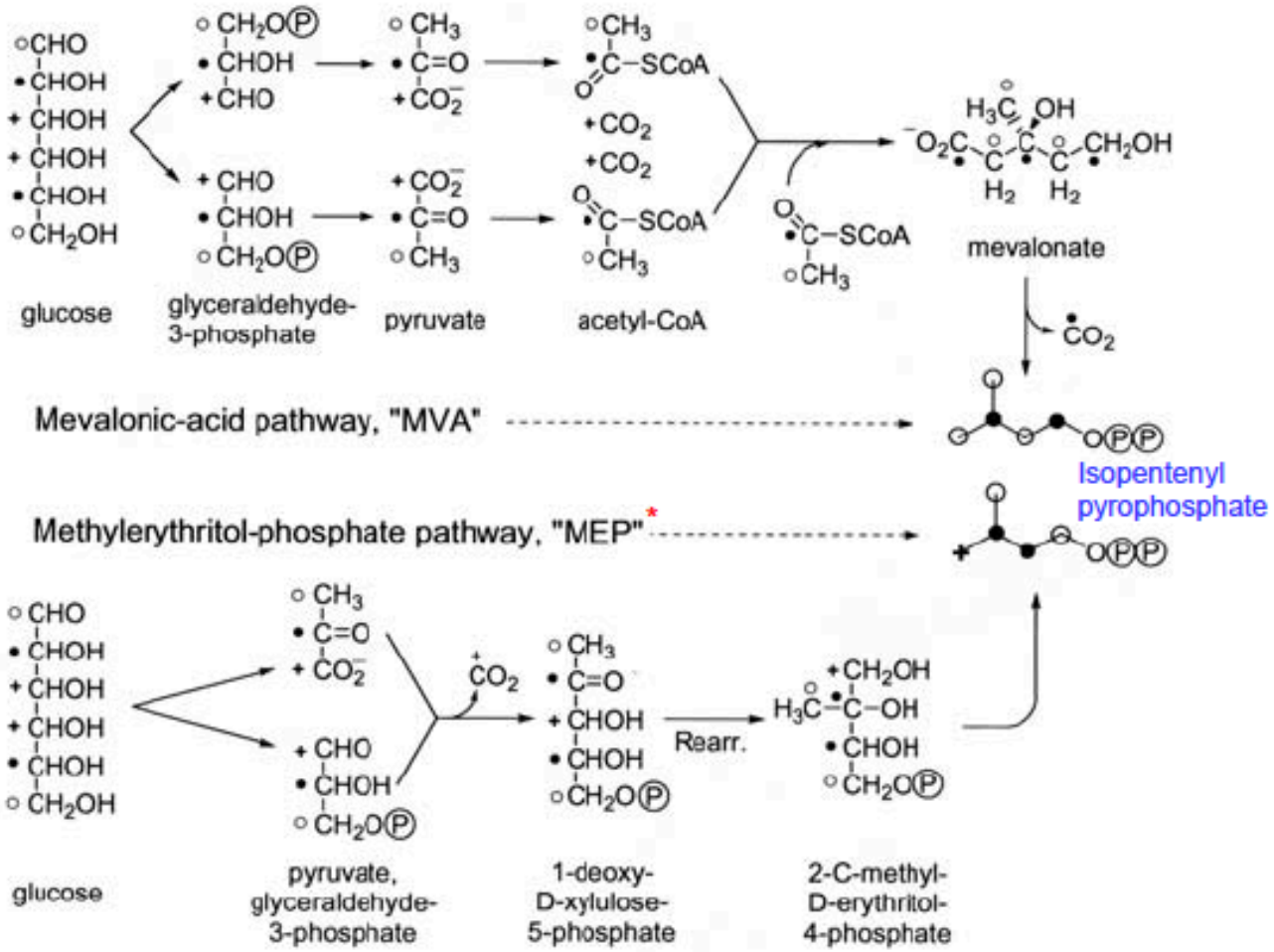






Averaged histogram representation for (a) C3 angiosperm and (b) C3 gymnosperm trees as well as (c) C3 and (d) C4 grasses of contents of n-$C_{26}$ to n-$C_{36}$ alkanes in leaf waxes (in % of compound class, normalised to the most abundant homologue, left Y-axis; black bars), overlain by averaged molecular stable carbon isotope data ($\delta^{13}C$, right Y-axis). ACL: mean average chain length of odd-carbon number n-alkanes (n-$C_{27}$ to n-$C_{35}$). $\delta^{13}C_{WMA}$: mean weighted mean average of carbon isotopic values of odd-carbon-numbered n-C27 to n-C35 alkanes. n: number of species used for the averaging of content data as well as of isotopic data.

Rommerskirchen et al.

# Pathways of isoprenoid synthesis: Isotopic implications



# Isotopic distribution within biomarkers

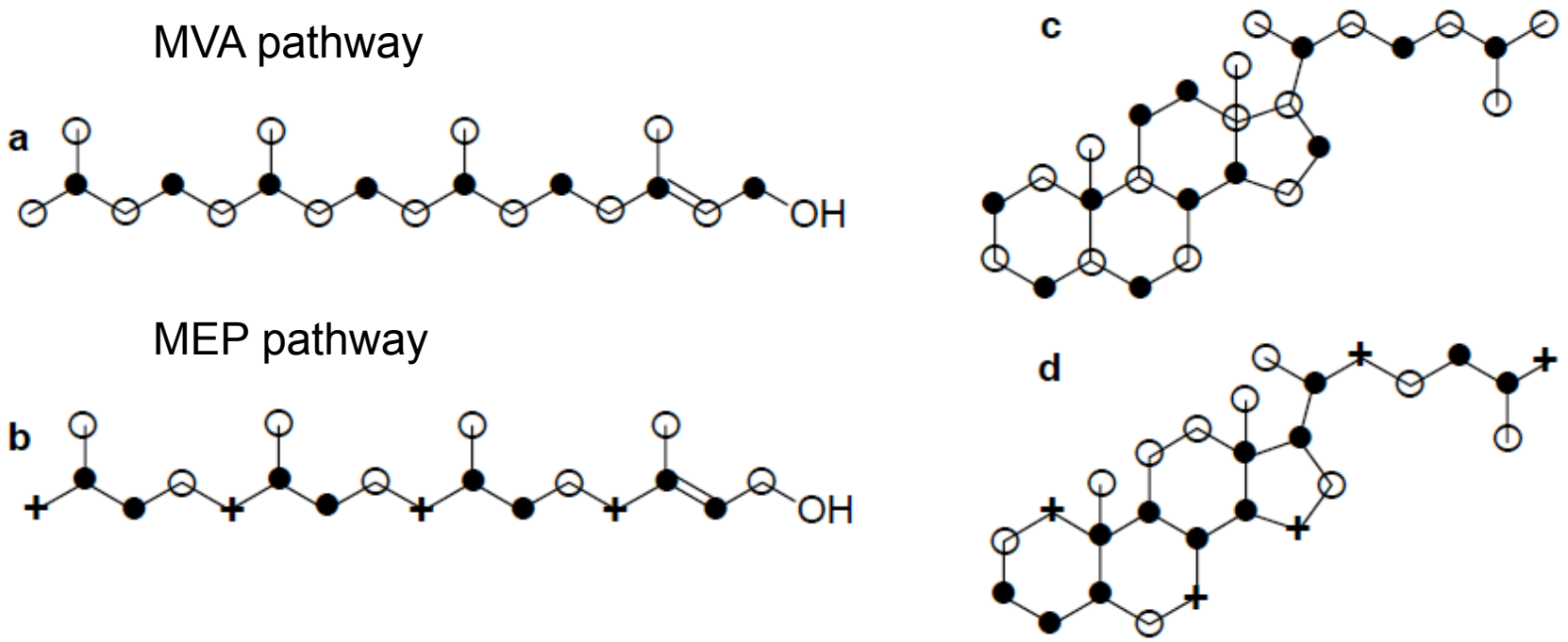
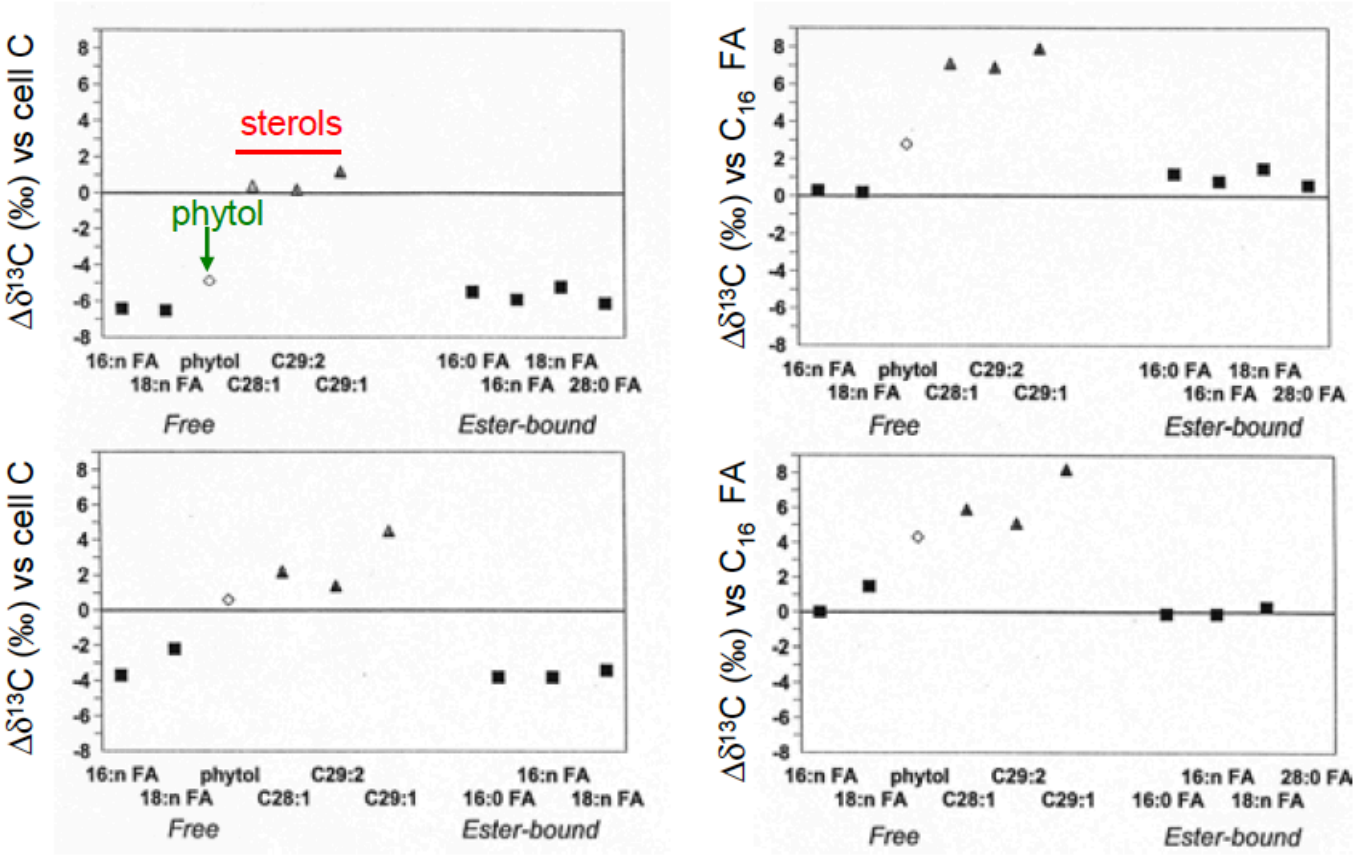



**Figure 29.** Relationships between the carbon positions in isopentenyl pyrophosphate and their sources. In the mevalonic-acid pathway, all five carbon positions in isopentenyl pyrophosphate derive from acetate and, in turn, from the C-1 + C-6 and C-2 + C5 positions of glucose. In the methyleryth-ritol-phosphate pathway, one carbon derives from the C-3 + C-4 position in glucose. The mapping of positions from precursors into products of the two pathways differs sharply, as indicated by struc-tures of acyclic and steroidal carbon skeletons based on the MVA (a, c) and MEP pathways (b, d).

# Stable carbon isotopic composition in *T. minimum* (freshwater green alga)

Continuous cultures




Batch cultures

# Carbon Stable Isotopes: Summary

*Carbon isotope values are measured my mass spectrometry relative to a carbonate standard*

*Most organic carbon values are depleted or more negative than the standard*

*Carbon isotopic values are effected by thermodynamic and kinetic isotope fractionation effects.*

*In photautotrophs, the CO2 fixation pathway has a large impact on the isotopic value, and Allows us to distinguish C3 and C4 plants.*

*C3 biomass has an $\delta$13C value of about -26‰ while C4 biomass is -13‰*

*In marine algae the relationship is more complex, due to C concentration mechanisms, Uptake of CO2 and bicarbonate, etc., but marine organic matter is generally about -21‰*

*For heterotrophs, "you are what you eat +1". Loss of light carbon*

*At the biomarker level, isotopic values depend on a multitude of factors, but Generally lipids are depleted relative to biomass.*

*There are isotopic variations within molecules*

*Stable isotopes in biomarkers are useful for discerning sources, and may be useful in reconstructing paleoenvironments*