Stable Carbon Isotope Geochemistry

Key Reading:

- *Fogel M.L. and Cifuentes L.A. (1993) Isotope fractionation during primary production. In Organic Geochemistry (Eds. M.H. Engel and S.A. Macko). Plenum Press, New York. pp 73-98.*
- *Hayes J.M. (1993) Factors controlling ^{13}C contents of sedimentary organic compounds: Principles and evidence. Mar. Geol. **113**, 111-125.*

Suggested Reading:

- Hayes J.M., Freeman K.H., Popp B.N. and Hoham C.H. (1990) Compound-specific isotopic analyses: A novel tool for reconstruction of ancient biogeochemical processes. *Org. Geochem.* **16**, 1115-1128.
- Fontugne M. and Duplessy J.C. (1978) Carbon Isotope Ratio of Marine Phytoplankton related to surface water masses. *Earth and Planetary Sci. Lett.* **41**, 365-371.
- Smith B.N and Epstein S. (1971) Two categories of $^{13}C/^{12}C$ ratios for higher plants. *Plant Physiol*. **47**, 380-384.
- O'Leary M.H. (1981) Carbon Isotope Fractionation in Plants. *Phytochem.* **20**, 553-567.
- Fry B. and Sherr E.B. (1984) ^{13}C Measurements as Indicators of Carbon Flow in Marine and Freshwater Ecosystems. *Contrib. Mar. Sci.* **27**, 13-47.
- Gearing P., Plucker F.E. and Parker P.L. (1977) Organic Carbon Stable Isotope Ratios of Continental Margin Sediments. *Mar. Chem.* **5**, 251-266.
- Bromley B.W., Hegeman G.D. and Meinschein W. (1982) A method for measuring Natural Abundance Intramolecular Stable Carbon Isotopic Distributions in Malic Acid. *Anal. Biochem.* **126**, 436-446.

Stable Carbon Isotopes

There are 2 stable isotopes of carbon:

Isotope	Abundance
^{12}C	98.89 %
^{13}C	1.11 %

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

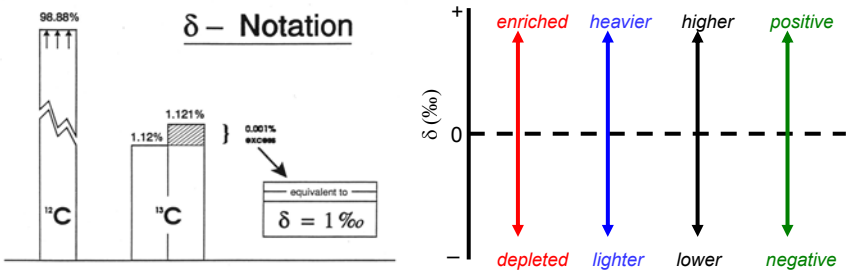
Nomenclature:

$$\delta^{13}C \text{ (permil, ‰)} = [(^{13}C/^{12}C)_{spl}/(^{13}C/^{12}C)_{std} - 1] \times 1000$$

Standard reference materials:

	$^{13}C/^{12}C$	Symbol
• PeeDee Belemnite (carbonate)	1.123×10^{-2}	PDB
• Solenhofen limestone	1.1218×10^{-2}	NBS-20

Notation and nomenclature



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:

$$^{13}CO_2(g) + H^{12}CO_3^-(aq) = {}^{12}CO_2(g) + H^{13}CO_3^-(aq)$$

- The fractionation factor, α, is expressed as:

$$\alpha HCO_3^-/CO_2 = (^{13}C/^{12}C)HCO_3^-/(^{13}C/^{12}C)CO_2$$

A related expression is the "difference fractionation factor"

$$\varepsilon \equiv \Delta^{13}C = \delta^{13}C_{product} - \delta^{13}C_{reactant}$$

2 types of isotope effect:

(i) Equilibrium isotope effects

(ii) Kinetic isotope effects

Equilibrium isotope effects

1. Rule of thumb - the heavy isotope (^{13}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:

$$^{13}CO_2(g) + H^{12}CO_3^-(aq) = {}^{12}CO_2(g) + H^{13}CO_3^-(aq)$$

$$K=\{[^{12}CO_2(g)][H^{13}CO_3^-(aq)]\} / \{[^{13}CO_2(g)][H^{12}CO_3^-(aq)]\}$$

- The major equilibrium isotope system affecting organic carbon isotope compositions is the inorganic carbonate buffer system. At seawater pH:

	$CO_2(g)$	=	$CO_2(aq)$	=	$H_2CO_3(aq)$	=	HCO_3^-	=	CO_3^{2-}
$\Delta^{13}C$		+1		+8		-1		-1.7	
$\delta^{13}C$	-7		-6		+2		+1		-0.5

- *N.B. The major fractionation effect is the hydration of CO_2.*
- (i.e. bicarbonate is enriched in ^{13}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}C and ^{12}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 ($\Delta^{13}C$) 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 (CO_2, CH_4)

- - Fixation of CO_2 by primary producers (photosynthesis)
- - Fixation of CO_2 by chemoautotrophs (sulfide oxidisers, methanogens)
- - Processing of intermediates in methanogenesis
- - Assimilation of C_1 compounds by methylotrophs

Multi-carbon substrates

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

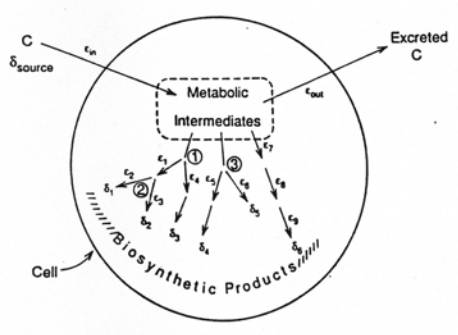


Fig. 2. An overview of a cellular carbon budget and of pathways leading to biosynthetic products. Kinetic isotope effects are denoted by ε and are arbitrarily numbered to indicate their independence. Isotopic compositions are denoted by similarly numbered δ terms. The circled numbers indicate branch points.

Isotope Fractionation during Photosynthesis, ε_P

In photosynthesis $^{12}CO_2$ is preferentially taken up relative to $^{13}CO_2$. There are two stages when kinetic isotope effects can occur:

1. Transport (diffusion) processes

- Gas phase diffusion (*i.e.* Atmospheric $CO_2 \rightarrow$ dissolved CO_2 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 CO_2 or HCO_3^-
  - Approx fractionation factor: 0.8 ‰ (relatively minor)

2. Chemical (Enzymatic) processes

- Four pathways:
  - (i) C_3 (Calvin-Benson)
  - (ii) C_4 (Hatch-Slack)
  - (iii) CAM
  - (iv) Bacterial

(i) The C_3 (Calvin-Benson) pathway

- Most common for terrestrial (vascular) plants and phytoplankton (also cyanobacteria).
- All trees use C_3 pathway

Characteristics:

- optimum growth temperature: 20-35°C
- CO_2 compensation point: 0.004%
- light saturation 3,000 ft.cdl
- max. photosynthetic rate: slow
- enzyme: Ribulose-1,5-biphosphate (RuBP) carboxylase-oxygenase ("RUBISCO")

  $\Delta^{13}CO_2$: -23 to -41 ‰.
  - ave. –27* ‰ for land plants
  - ave. –25* ‰ for unicellular phytoplanklton

**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:

$$6CO_2 + 12\ NADPH + 18\ ATP \rightarrow C_6H_{12}O_6 + 12\ NADP^+ + 18\ ADP$$

(energy and reduction power come from $h\upsilon$ splitting of H_2O mediated by chlorophyll)

Carbon isotopic fractionation during C_3 photosynthesis

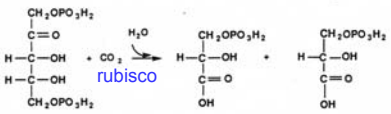
Model describing the isotopic fractionation, Δ, in C_3 plants:

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

where

- a is the isotope effect associated with diffusion of CO_2 into the plant (~ 0.8 ‰)
- 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)

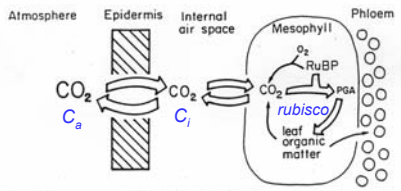


Figure 2. Important steps in CO_2 fixation during C_3 photosynthesis. Sizes of arrows indicate the relative fluxes through the various steps (including the reverse steps) according to the best models available. Sizes of symbols reflect relative concentrations of CO_2 at various stages.

(ii) The C_4 (Hatch-Slack) pathway

- Less common for vascular plants
- Exceptions: sugar cane, corn, bamboo (typical of plants in hot arid climates)
- Tropical grasses, desert plants, salt marsh plants.
- The adaptation allows maximum CO_2 fixation per unit loss of water.

Characteristics:

- optimum growth temp.: 35°C
- CO_2 compensation point: 0.0004% (1/100th of today's atmosphere)
- light saturation: 10,000 ft cdl.
- max. photosynthetic rate: fast
- enzyme: phosphoenyl pyruvate (PEP) carboxylase
  - *N.B. no competing oxygenase activity*

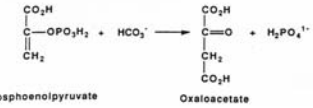
$\Delta^{13}CO_2$: -0.5 to -3.6 ‰ (ave. -2.2 ‰).

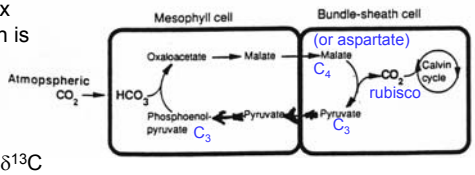
(ii) The C_4 (Hatch-Slack) pathway

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.

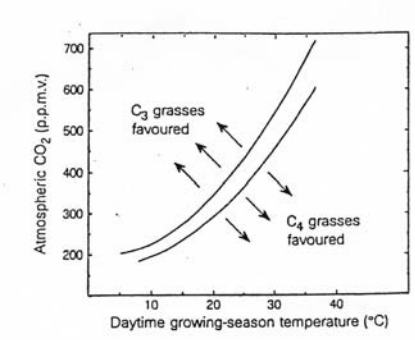
The smaller (ca. 2 ‰) isotope effect associate with the PEP carboxylase enzyme give C_4 plants more positive $\delta^{13}C$ values (-8 to -18 ‰).

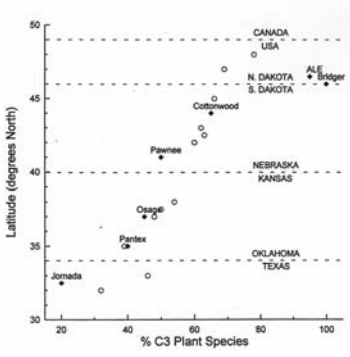
Formation of oxaloacetate (C_4) from phosphenolpyruvate by the PEP carboxylase reaction





Environmental Conditions favoring C_4 photosynthesis





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)
- ϕ 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. Recently it has been shown that some algae (diatoms) can use a C_4-like pathway (Reinfelder et al., 2000, Nature 407, 996-999).

(iii) The CAM (crussulacean acid metabolism) pathway

- Used by succulents – cacti, crassulaceae
- Similar to C_4 pathway, but different spatial and temporal packing.
- Plants using this pathway have intermediate isotopic compositions between C_3 and C_4.
- Isotopically speaking, marine algae also generally fall between C_3 and C_4.
- 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 CO_2 from CAM plants

(iv) Anoxygenic bacterial photosynthesis

- Doesn't produce O_2; H_2 taken from H_2S instead of H_2O:

$$CO_2 + 2H_2S \rightarrow CH_2O + S_2 + H_2O$$

- Some bacteria (e.g. purple photosynthetic bacteria) use RuDP pathway to fix CO_2 whereas others use an inversion of the Acetyl-CoA decarboxylation reaction - "reverse TCA cycle" (green photosynthetic bacteria).
- The latter is important in Black Sea (see later lecture) - green sulfur bacteria (Chlorobiaceae) - anaerobic photoautotrophs.

$$\text{Acetyl-CoA} + CO_2 \xrightarrow[\text{feridoxin}]{} \text{pyruvate} + \text{CoA}$$

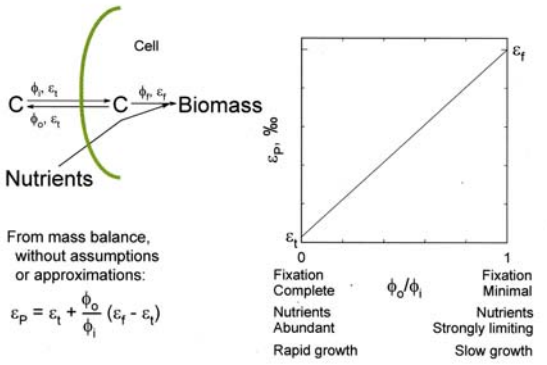
- fractionation factors range from -10 to -30 ‰

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 $[CO_2]_{aq}$ 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.
- Most 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:

$$\Delta = d + b_3 \times (F_3/F_1)$$

- Where:
  - d is the equilibrium isotope effect between CO_2 and HCO_3^-
  - b_3 is the isotopic fractionation associated with carboxylation (by RUBISCO)
  - F_3/F_1 is the ratio of CO_2 leaking out of the cell to the amount inside the cell.



Active transport (pumping) of DIC into algal cells

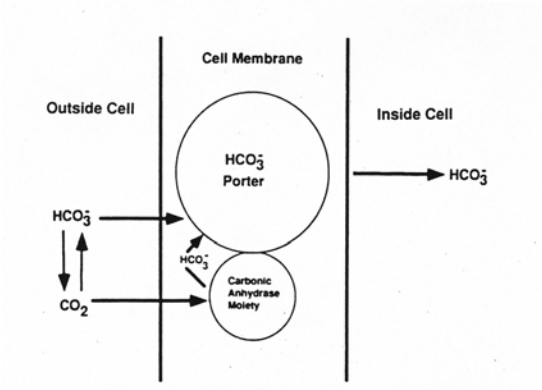
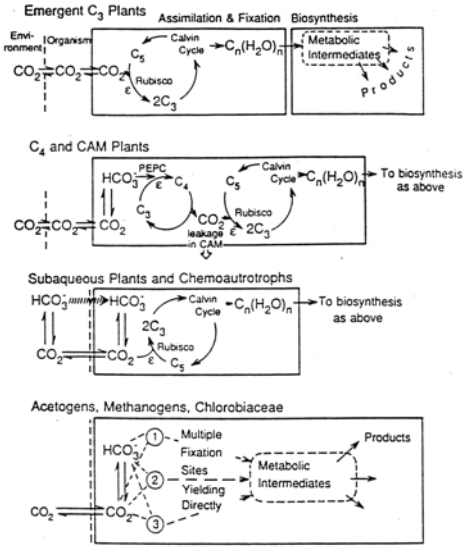


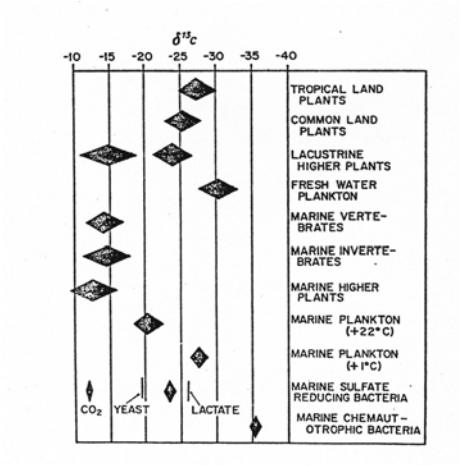
Figure 6. Model for dissolved inorganic carbon transport.

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 fractionation in aquatic photoautotrophs

Other Considerations:

$\delta^{13}C$ of inorganic carbon source

- In general the $\delta^{13}C$ of a photosynthetic organism will be the sum of the $\delta^{13}C$ of the inorganic source and the $\Delta^{13}C$ of the enzymatic fractionation:

$$\delta^{13}C_{organism} = \delta^{13}C_{DIC} + \Delta^{13}C_{enzyme}$$

- For marine phytoplankton the effects of temperature on the marine DIC pool and on the enzymatic system used to fix CO_2 are difficult to separate and isotopic trends with temperature are complex.

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:

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

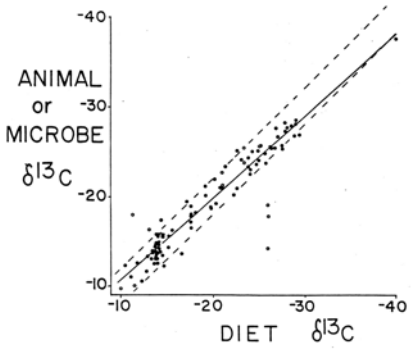
where:

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

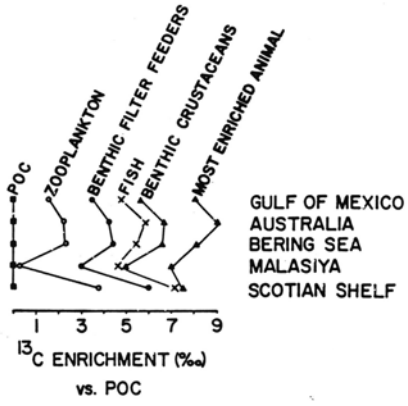
Before a carbon atom can be lost a carbon-carbon bond must be broken (in most cases).

- ^{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

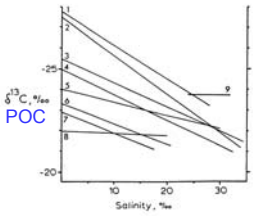
- *Potential problems:*
- Individual variability in $\delta^{13}C$ averages 1 to 2 ‰ (masks assimilation effect)
- Results are often tissue or biochemical dependent.

- **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 what we study*

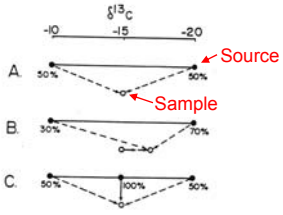
Stable Carbon Isotopes as Source Indicators

- 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
- Reasons:
  - End-members are unknown
  - End-members although known are temporally variable
  - There are more than 2 end-members

River-ocean transects



Isotope mixing models



A. 2 sources (50:50)
B. 2 sources (30:70), but δ 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.

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

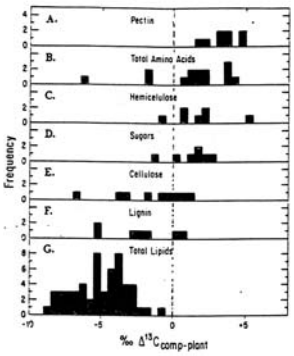
– where f = mole fraction as C

- In general: 1‰ 5‰

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

Increasingly ^{13}C depleted

Isotopic variations between higher plant biochemicals



Isotopic variations in vascular plant leaf biochemicals

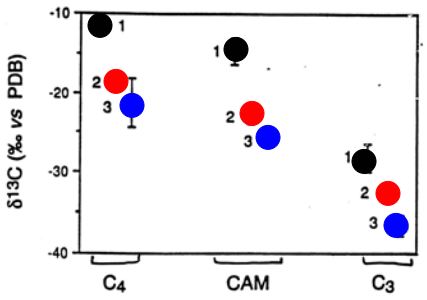
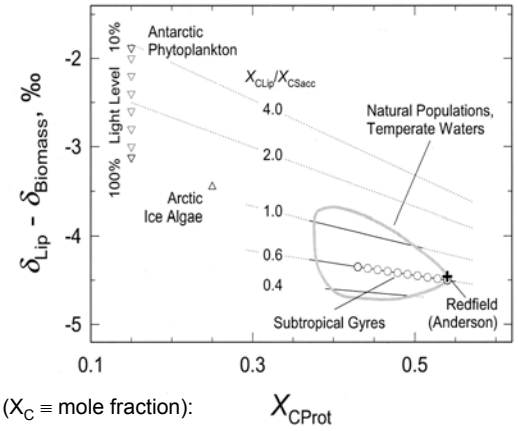


Fig. 2. Average $\delta^{13}C$ (‰ vs PDB) values for total tissue (1), total surface lipid extracts (2) and weighted mean *n*-alkanes (3) for each of the three plant groups (C_4, CAM and C_3). Error bars represent the standard deviation of the measurements for each fraction for the given plant group.

- Total tissue
- Total lipid extract
- Total *n*-alkanes

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:

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

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

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).

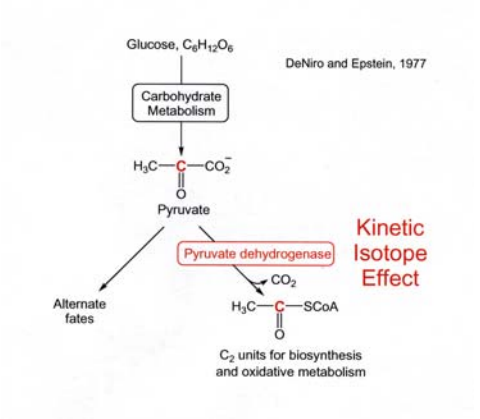
- For lipids:
  - Acetogenic lipids (based on acetate units)
  - Isoprenoid lipids
    - Mevalonic-acid pathway
    - Methylerythritol phosphate pathway (only recently recognized).

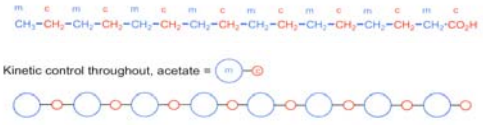
Carbon Isotopic distributions within molecules

- 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

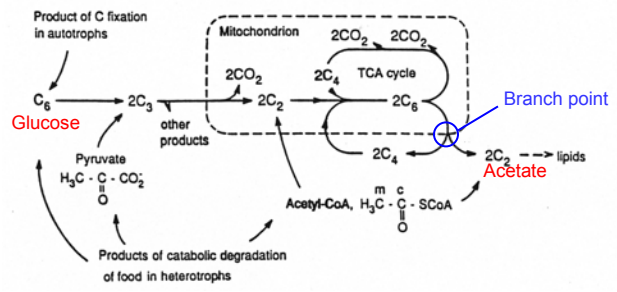
  (b) the biochemical pathway of fatty acid formation.



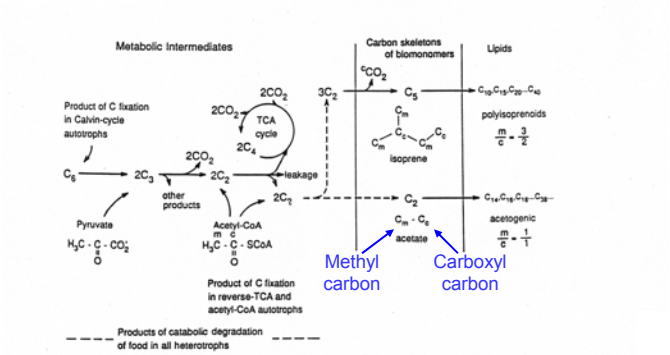


Pathways of lipid biosynthesis in eukaryotic organisms

Complexity is introduced by presence of subcellular organelles. Since acetate cannot pass through mitochondrial wall, C_2 units produced there by decarboyxlation of pyruvate are packaged as C_6 species (citrate) for export. Formation and subsequent conversion of C_6 to C_2 units provides possibilities for isotopic fractionation during biosynthesis of lipids.

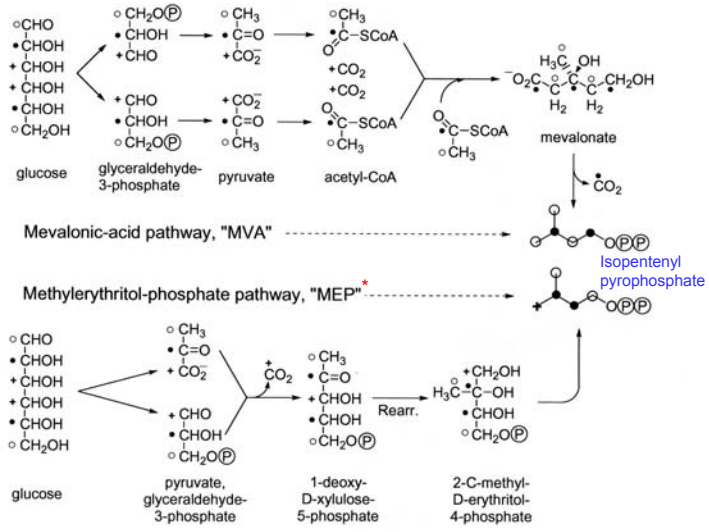


Pathways of lipid biosynthesis in prokaryotic organisms



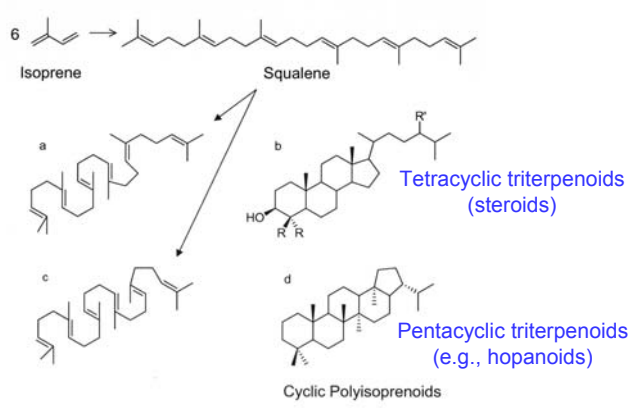
. 8. Pathways of lipid biosynthesis in prokaryotic organisms. In heterotrophs, any of the indicated multi-carbon organic ıpounds might derive from the food source. In Calvin-Cycle autotrophs the effective internal source of organic carbon is a C_6 ɔohydrate. For organisms using the reverse-TCA or acetyl-CoA systems of carbon fixation, the methyl and carboxyl positions of ɔyl-CoA will derive from two different fixaton sites. The letters m and c denote positions in the C_5 and C_2 biomonomers that 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

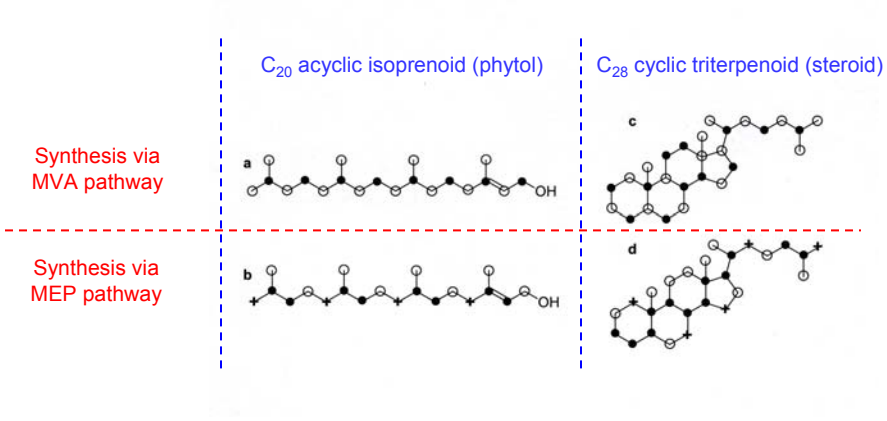


*aka Deoxyxylulose pathway

Synthesis of cyclic isoprenoids (triterpenoids)



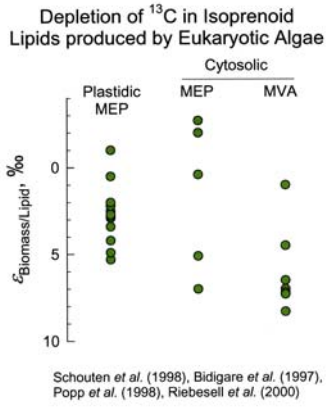
Isotopic distribution within isoprenoids



Factors bearing on isotopic fractionation in aquatic photoautotrophs

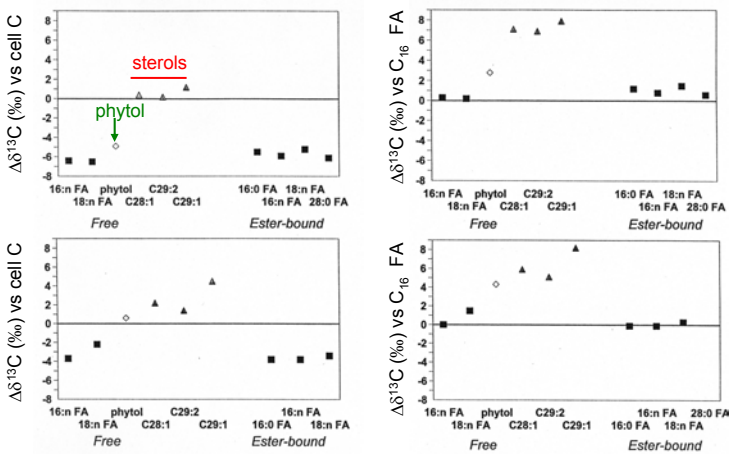
	Isoprenoid biosynthesis		Oxidative pentose phosphate pathway in plastid?
Organisms	C_{20}, C_{40}	C_{15}, C_{30}	
"Normal"	MEP	MVA	no
Green Algae	MEP	MEP	yes
Euglenoids	MVA	MVA	no
Cyanobacteria	MEP	MEP	n.a.

C_{20} and C_{40} generally plastidic (formed in chloroplast)
C_{15} and C_{30} commonly cytosolic
MEP = methylerythritol-phosphate pathway
MVA = mevalonic acid pathway



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

Continuous cultures



Batch cultures