Pagani, M., Authur, M.A. and Freeman, K.H. 1999. Miocene evolution of atmospheric carbon dioxide. *Paleoceanography* **14**: 273-292.

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

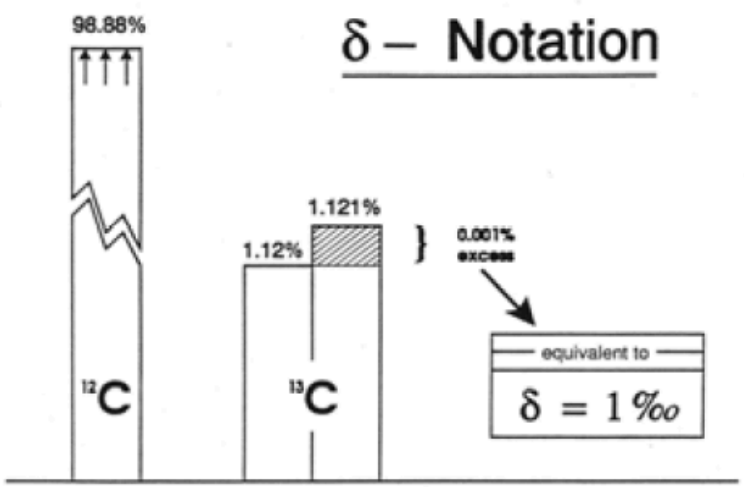
Nomenclature:

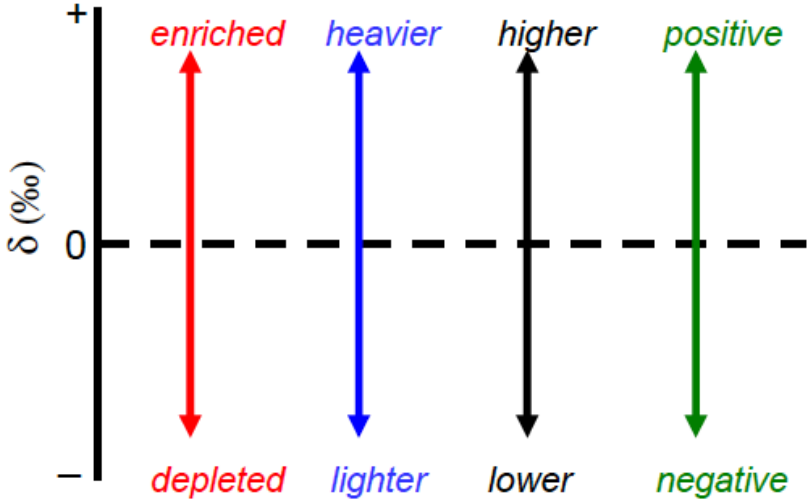
$$\delta^{13}C \text{ (per mil, ‰)} = [(^{13}C/^{12}C) \text{ sample}/ ((^{13}C/^{12}C) \text{ standard} - 1] \times 1000$$

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





Isotope fractionation effects

Physical, chemical, and biological processes lead to isotopic differences between reactants and products. These isotopic differences are conveniently expresses as *isotopic fractionations*.

Isotopic fractionations are measured by their fractionation factor, which can take several forms:

Δ (a/b) = $\delta\ ^{13}C(a) - \delta^{13}C(b)$

α(a/b): R_a/R_b where $R = {}^{13}C/{}^{12}C$

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

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

$$\alpha(HCO_3^-/CO_2) = R\ (HCO_3^-)/R\ (CO_2)$$

ε(a/b) = (α(a/b) -1) x1000

$\varepsilon(a/b) \equiv \Delta \equiv \delta\ ^{13}C(a) - \delta^{13}C(b)$

There are two types of isotope effects:

Thermodynamic isotope effects
Kinetic isotope effects

Equilibrium isotope effects

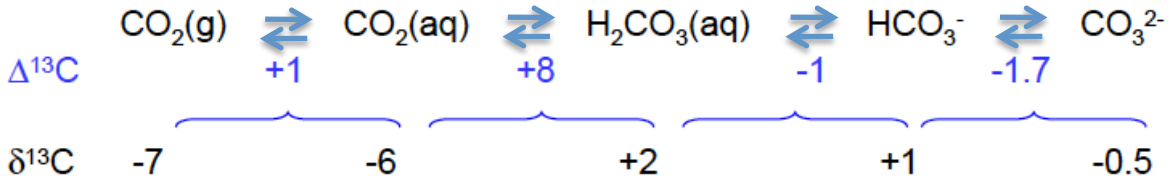
1. Rule of thumb - the heavy isotope (^{13}C) is concentrated in the chemical compound in which it is bound most strongly.
2. The expression for the isotopic exchange reaction is written just as for any chemical reaction and the equilibrium constant (K) is determined in the same way.

- 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 main equilibrium isotope system affecting organic carbon isotope compositions is the inorganic carbonate buffer system.



- *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 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 → 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

Fractionation during biosynthesis

Biosynthesis occurs through several different pathways, often with branch-points with common or unique intermediates. "upstream" isotopic fractionation will effect "downstream values.

Inorganic carbon transport and CO_2 accumulation in higher plants (top) and eukaryotic algal cells (bottom)

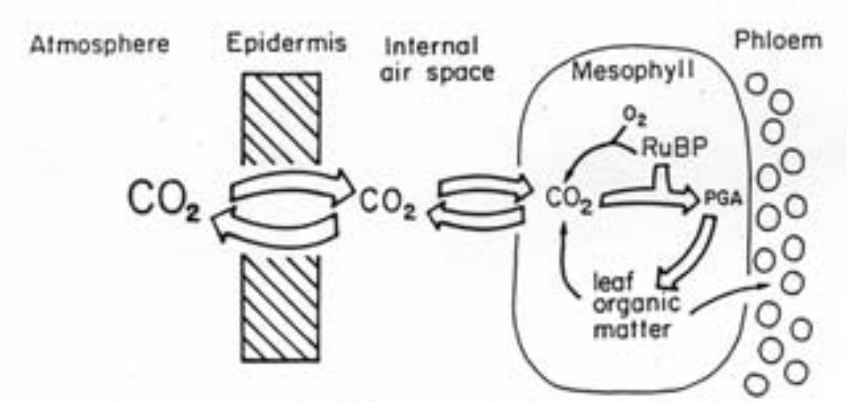
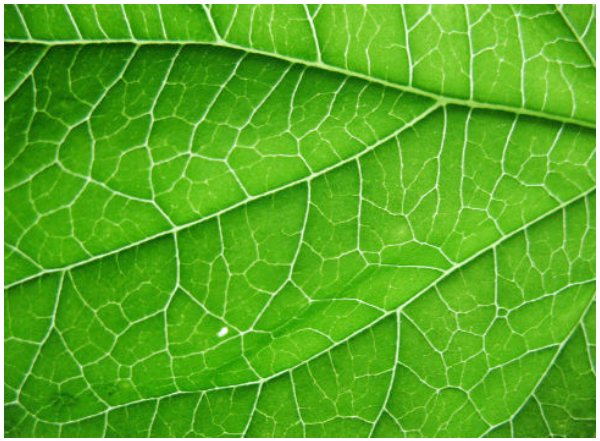


Figure 2. Important steps in CO_2 fixation during C_3 photosynthesis. Sizes of arrows indicate the relative fluxes through the various steps (including the reverse steps) according to the best models available. Sizes of symbols reflect relative concentrations of CO_2 at various stages.



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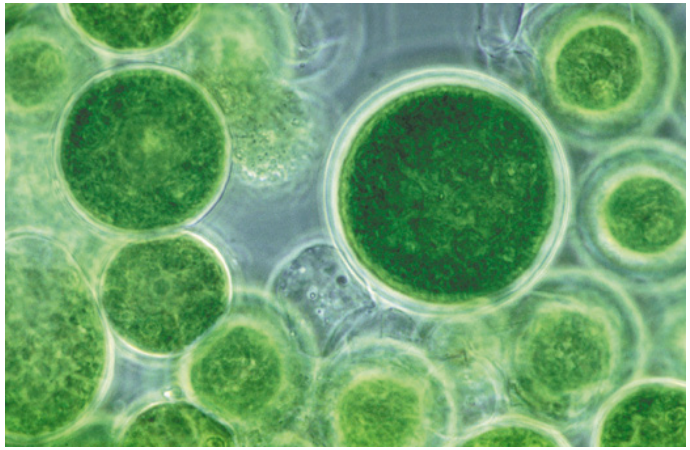

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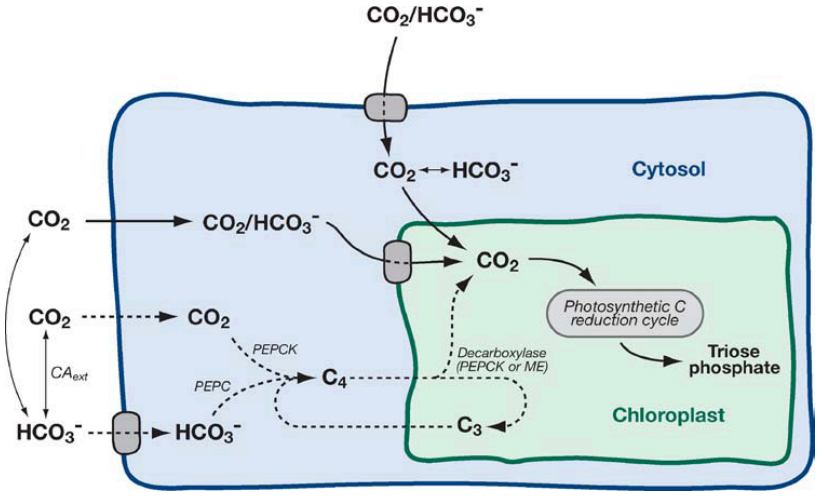


From Giordono et al. 2005 Ann Rev Plant Biology

(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 hυ splitting of H_2O mediated by chlorophyll)

Carbon isotopic fractionation during C_3 photosynthesis

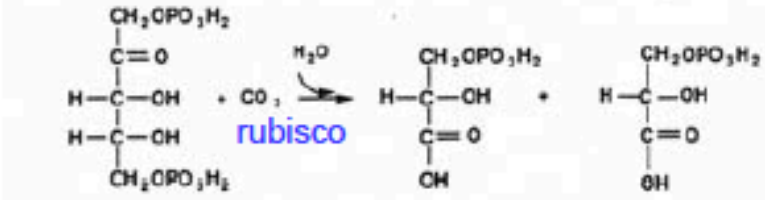
Model describing the isotopic fractionation, Δ, in C_3 plants:

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

where

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

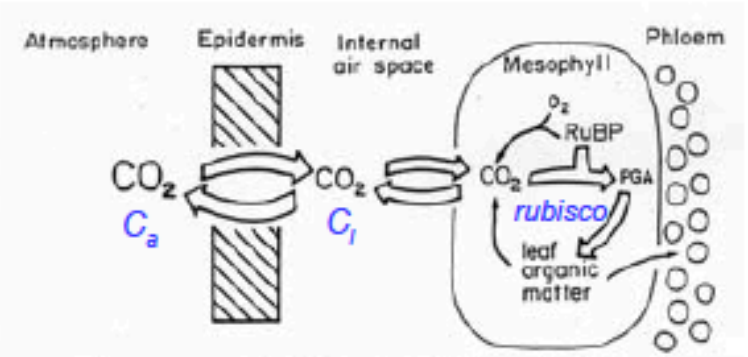


Figure 2. Important steps in CO_2 fixation during C_3 photosynthesis. Sizes of arrows indicate the relative fluxes through the various steps (including the reverse steps) according to the best models available. Sizes of symbols reflect relative concentrations of CO_2 at various stages.

(ii) The C_4 (Hatch-Slack) pathway

- 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 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*

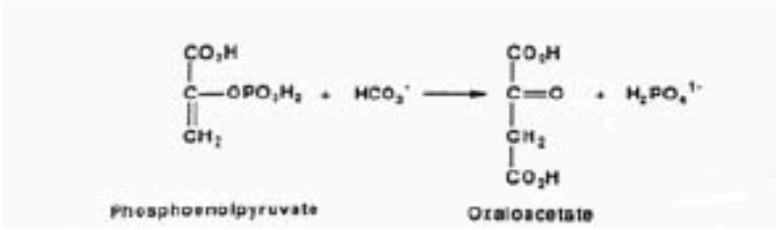
$\Delta^{13}CO_2$: -0.5 to -3.6 ‰ (ave. -2.2 ‰).

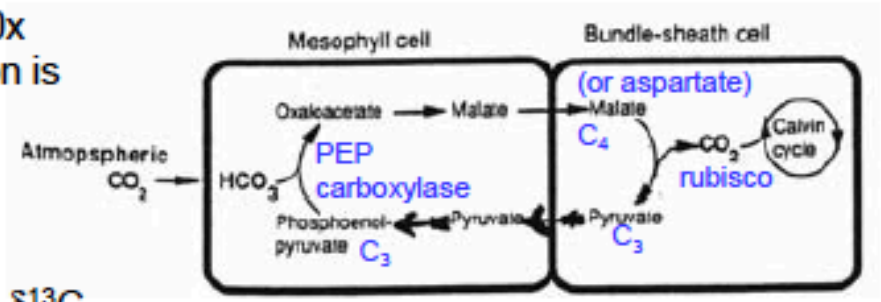
(ii) The C_4 (Hatch-Slack) pathway

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.

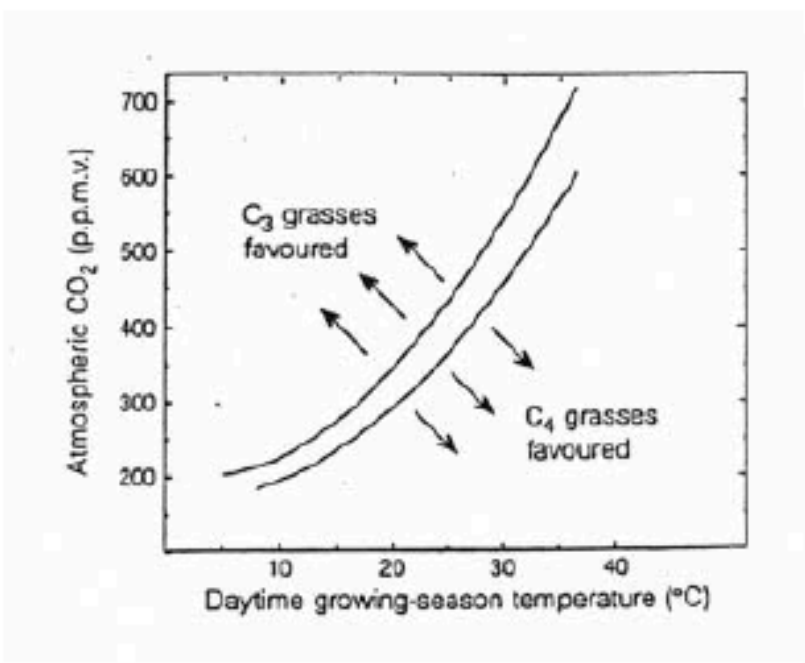
The smaller (ca. 2 ‰) isotope effect associate with the PEP carboxylase enzyme give C_4 plants more positive $\delta^{13}C$ values (-8 to -18 ‰).

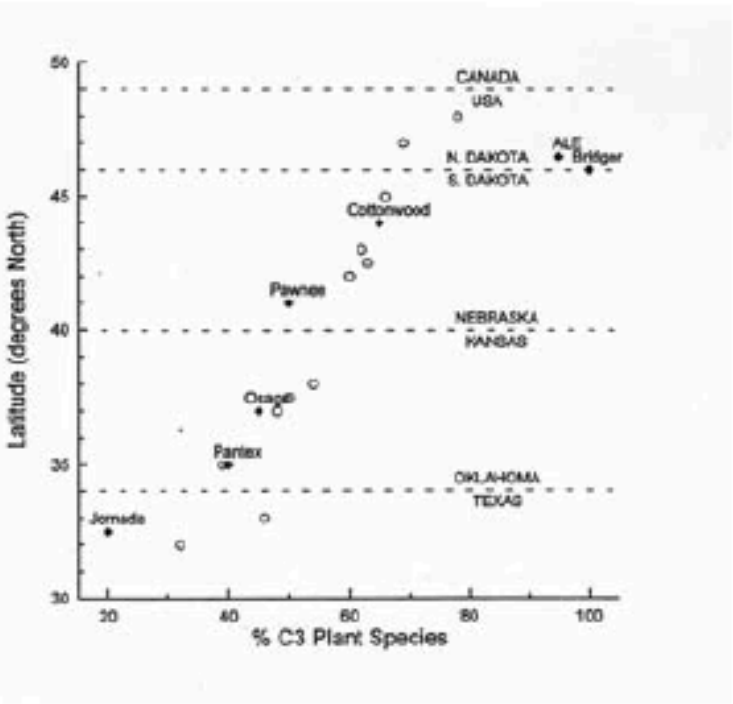
Formation of oxaloacetate (C_4) from phosphenolpyruvate by the PEP carboxylase reaction



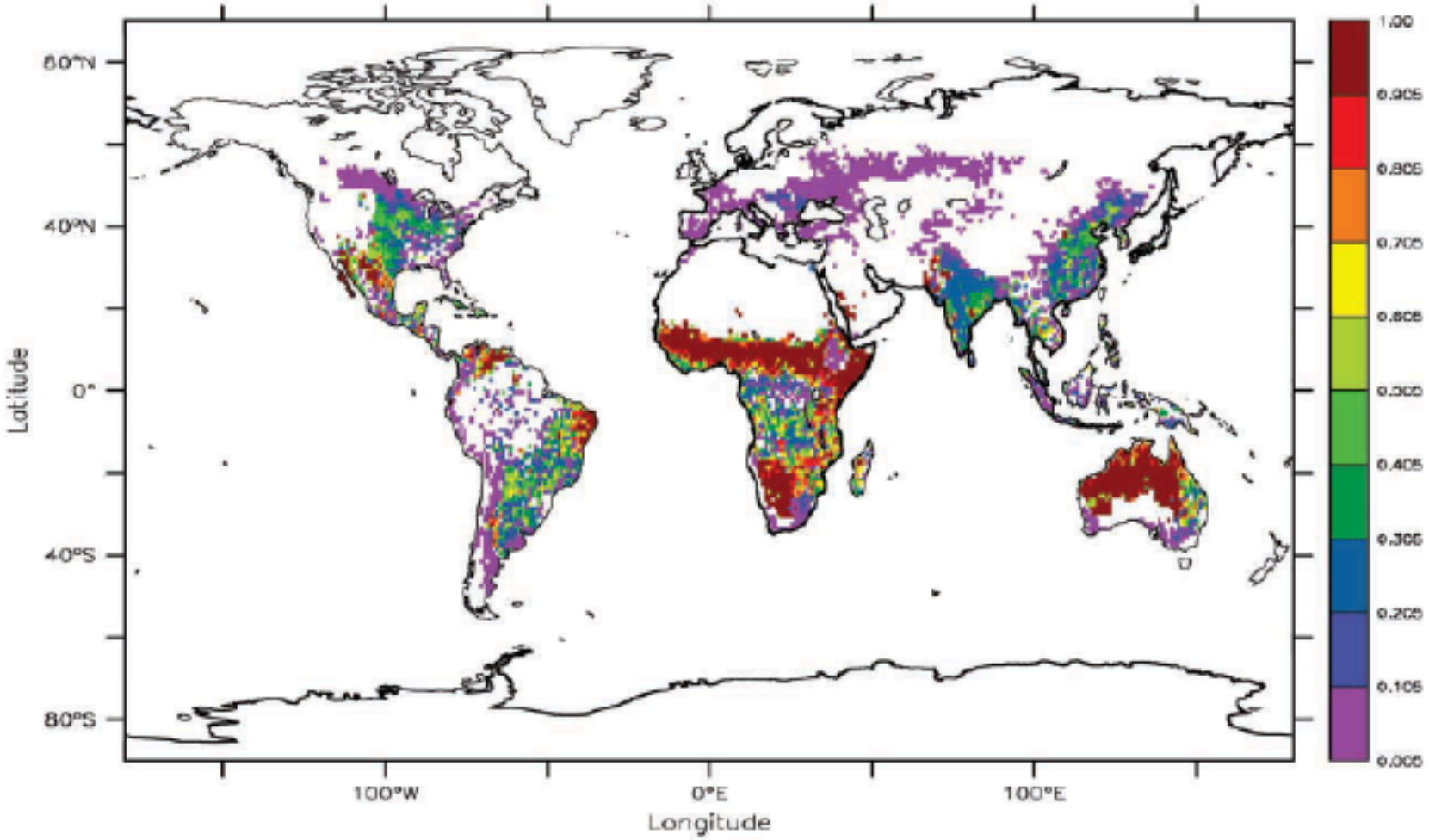


Environmental Conditions favoring C_4 photosynthesis





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)
- 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.

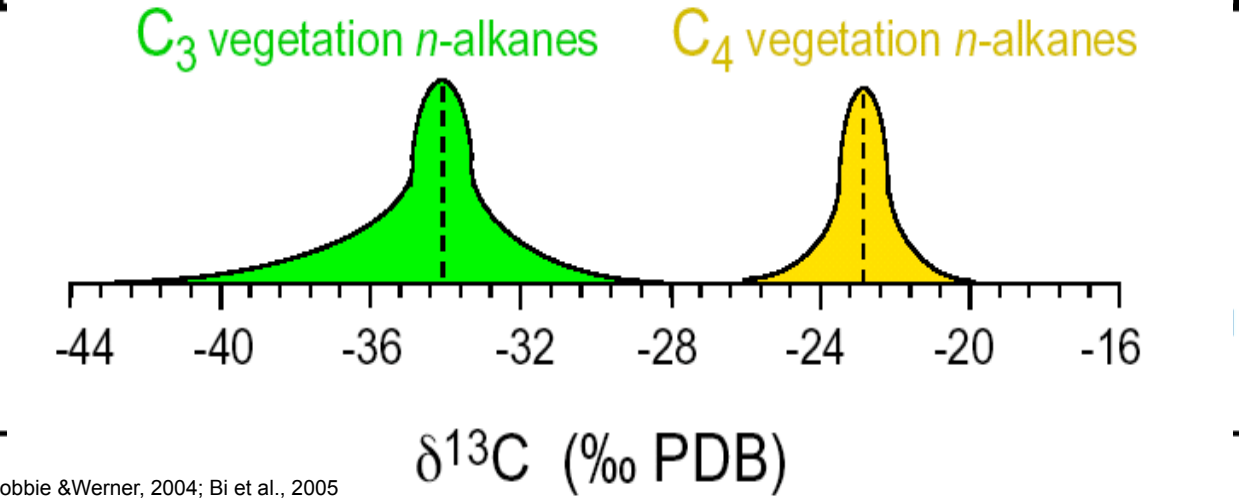
N.B. It has been shown that some algae (diatoms) can use a "C_4-like" pathway, i.e., possess CO_2 concentrating mechanisms (Reinfelder et al., 2000, Nature 407, 996-999).

(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

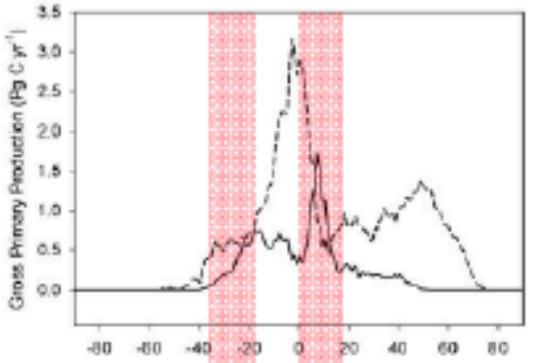
Isotopic ranges ($\delta^{13}C$, permil) for terrestrial plant biomass and plant wax *n*-alkanes

	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

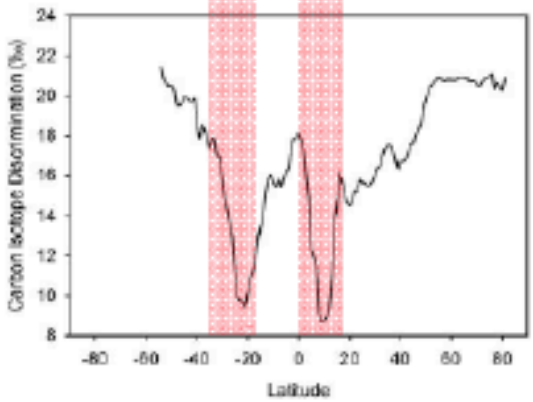


Collister et al., 1994; Hobbie &Werner, 2004; Bi et al., 2005

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.



Assimilation-weighted carbon isotope discrimination by latitude in per mil (‰).



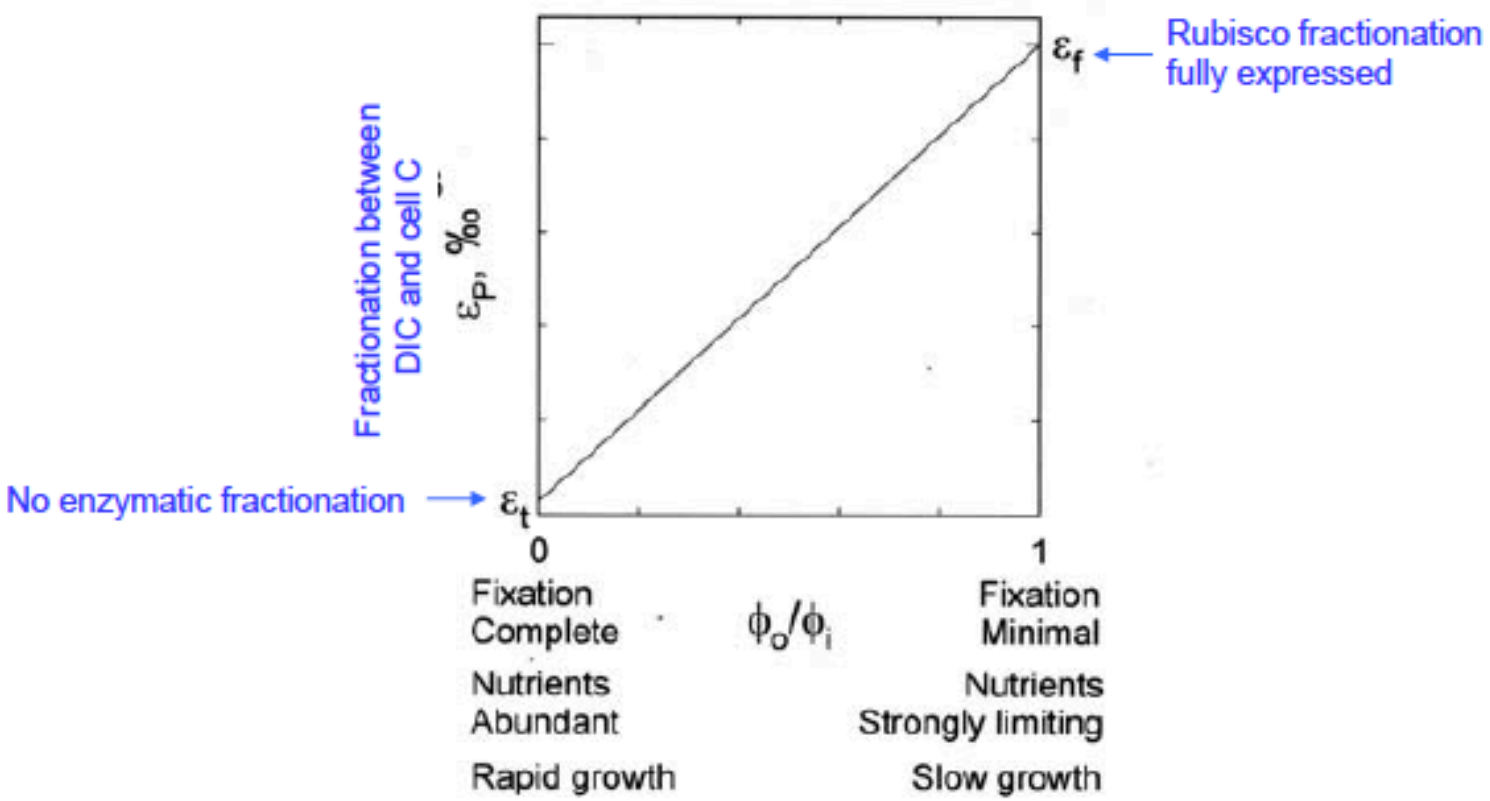
Still et al., 2003

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:

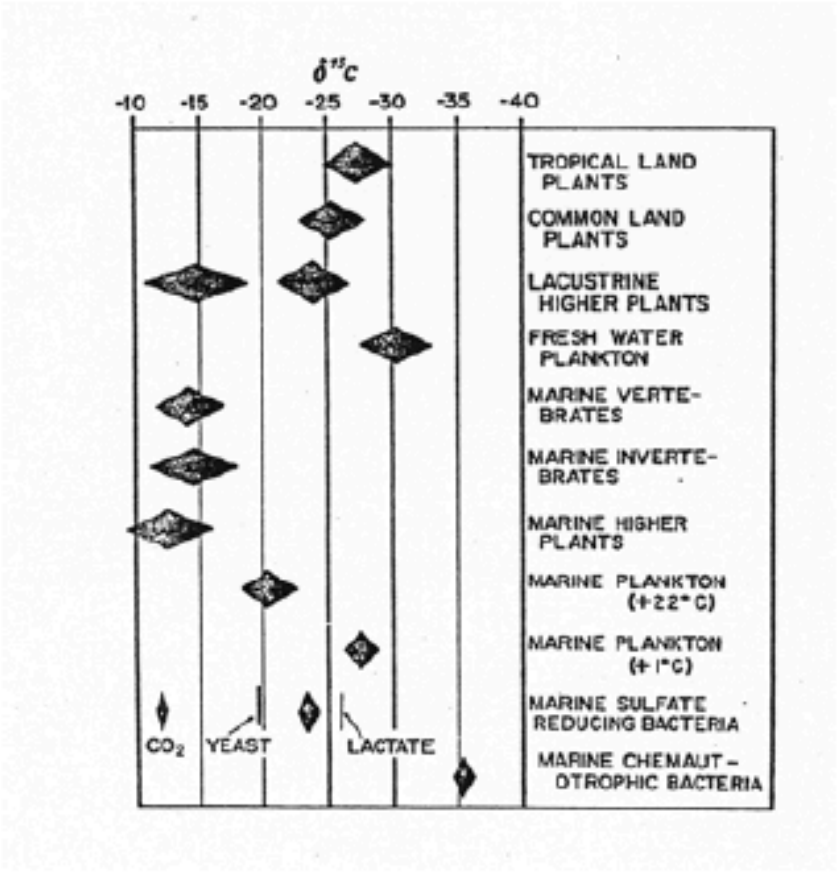
$$\Delta = d + b \times (\phi_o / \phi_i)$$

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



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

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:

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

- 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:

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

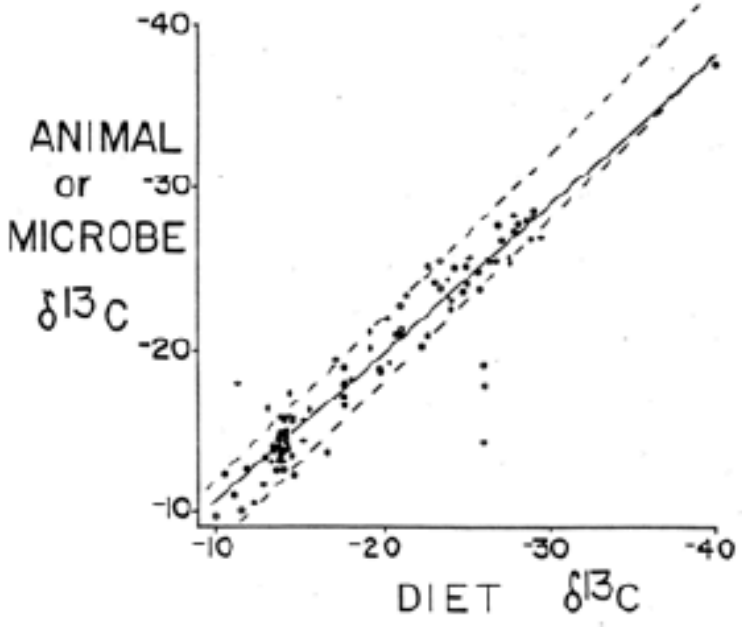
where:

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

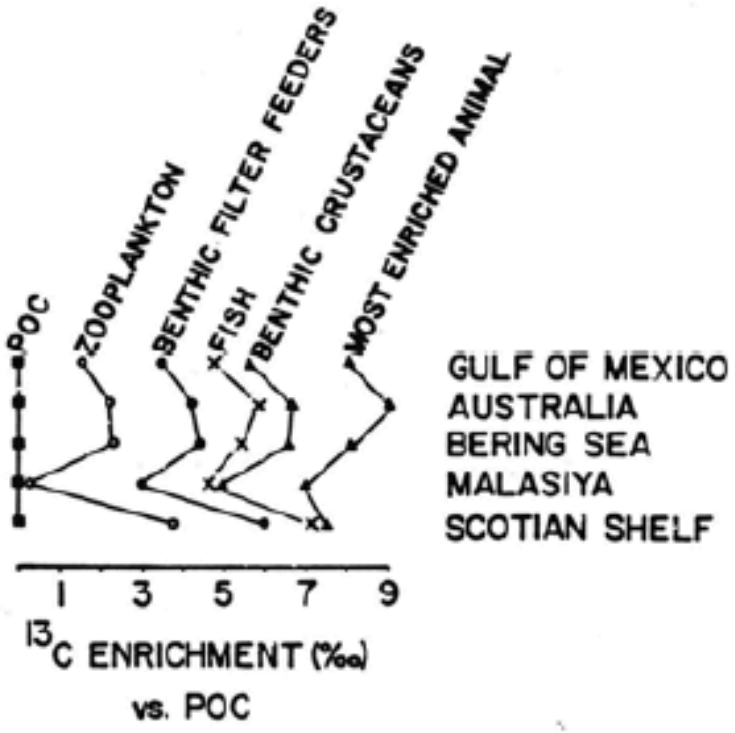
Before a carbon atom can be lost a carbon-carbon bond must be broken (in most cases).

- ^{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*

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.

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

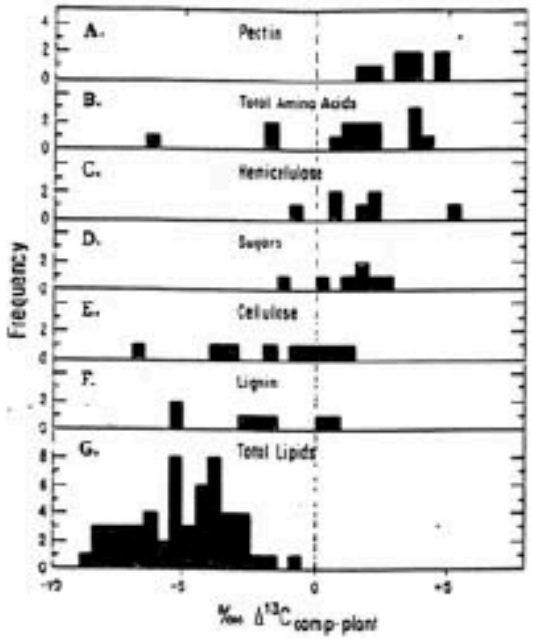
– where f = mole fraction as C

- In general: 1‰ 5‰

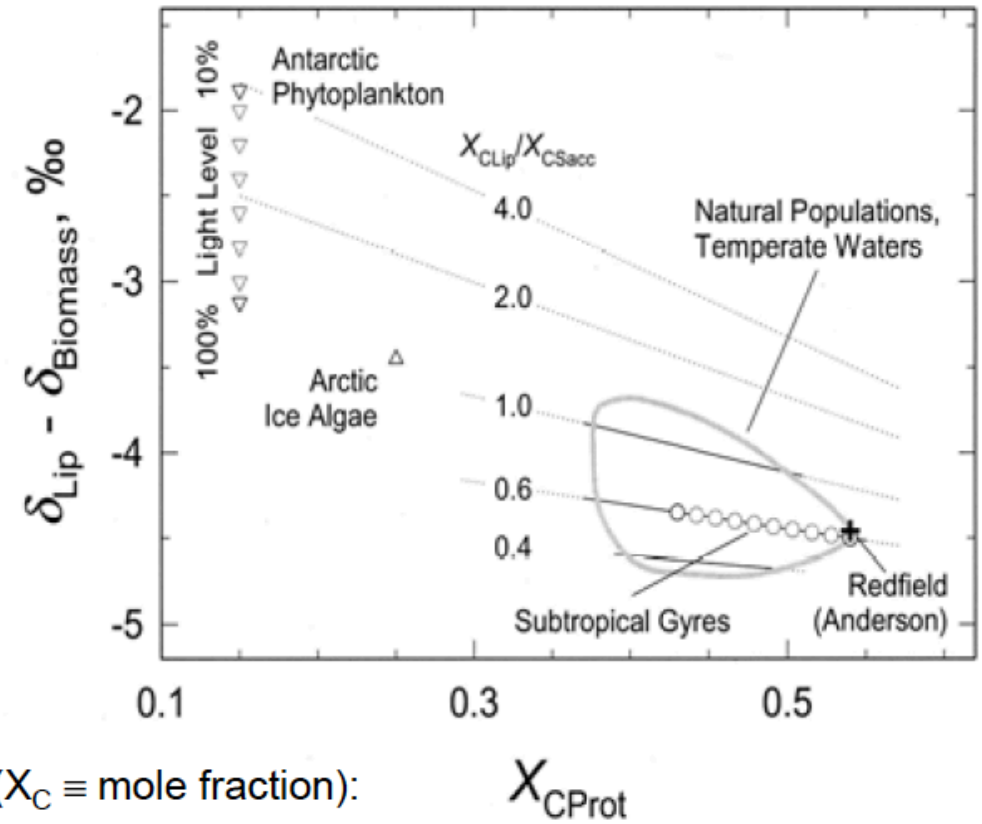
$$\delta_{PS} > \delta_{NA} \approx \delta_{Prot} > \delta_{Lipid}$$

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:

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

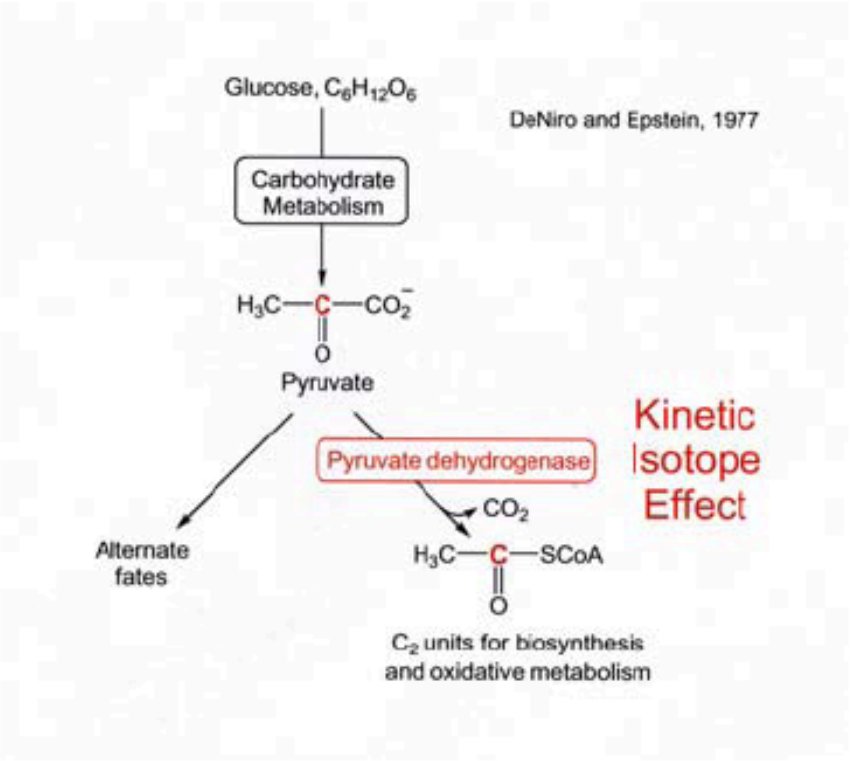
$\delta_{na} \approx \delta_{prot}$, $\delta_{prot} - \delta_{sacc} \approx -1$‰, $\delta_{lip} - \delta_{sacc} = -6$ ‰

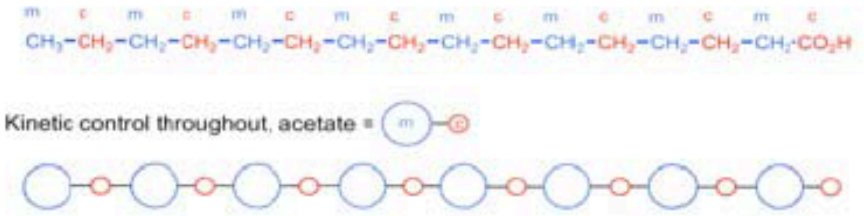
Carbon Isotopic distributions within molecules

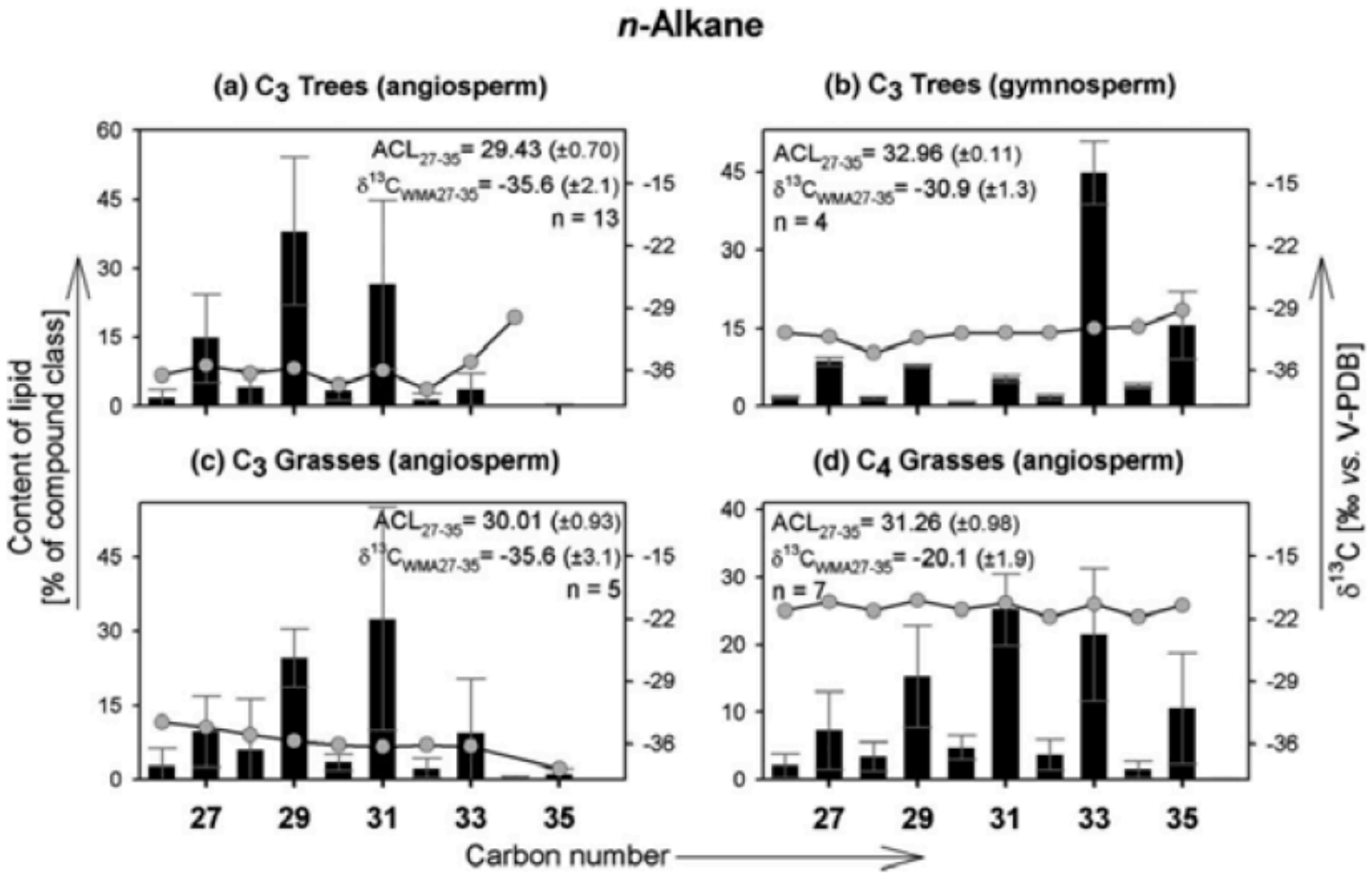
- Glucose is considered to be isotopically homogeneous
- Lipids exhibit sawtooth $\delta^{13}C$ distributions down linear carbon chains with the carboxyl-derived carbons from the acetyl-coA being about 6 ‰ lighter than the methyl-derived carbons.
- Monson and Hayes (1982) demonstrated alternating isotope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

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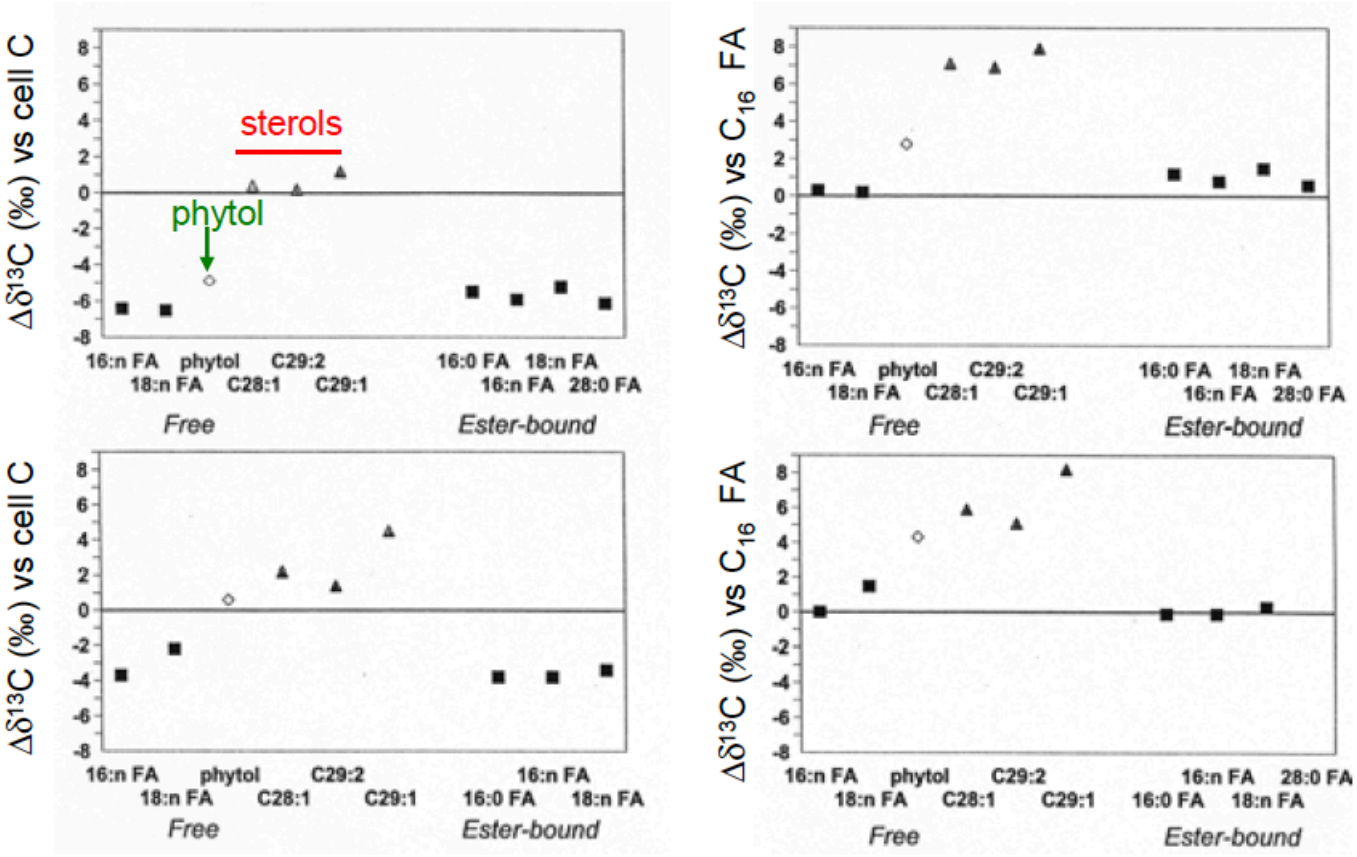
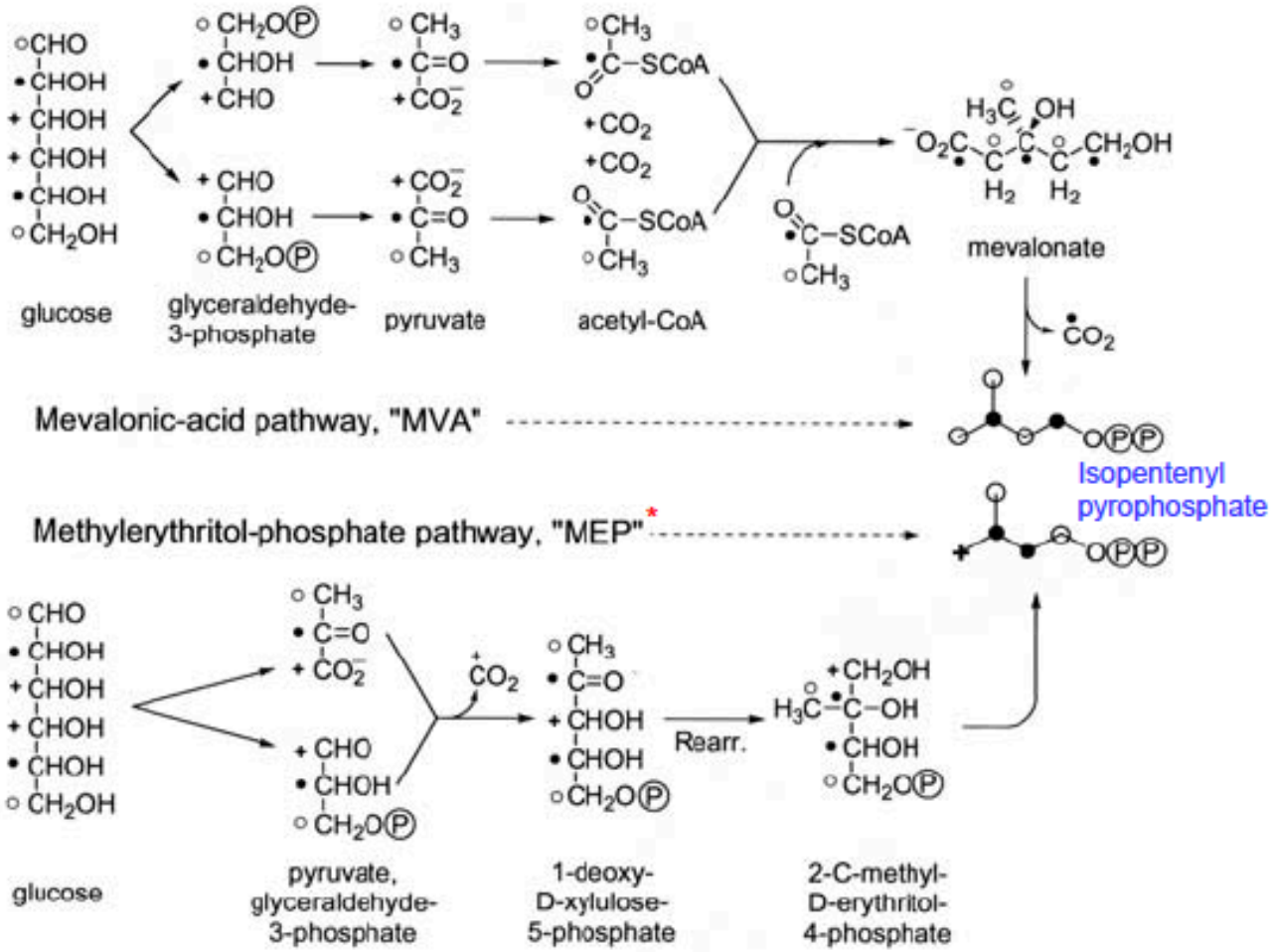
Stable carbon isotopic composition in *T. minimum* (freshwater green alga)

Continuous cultures



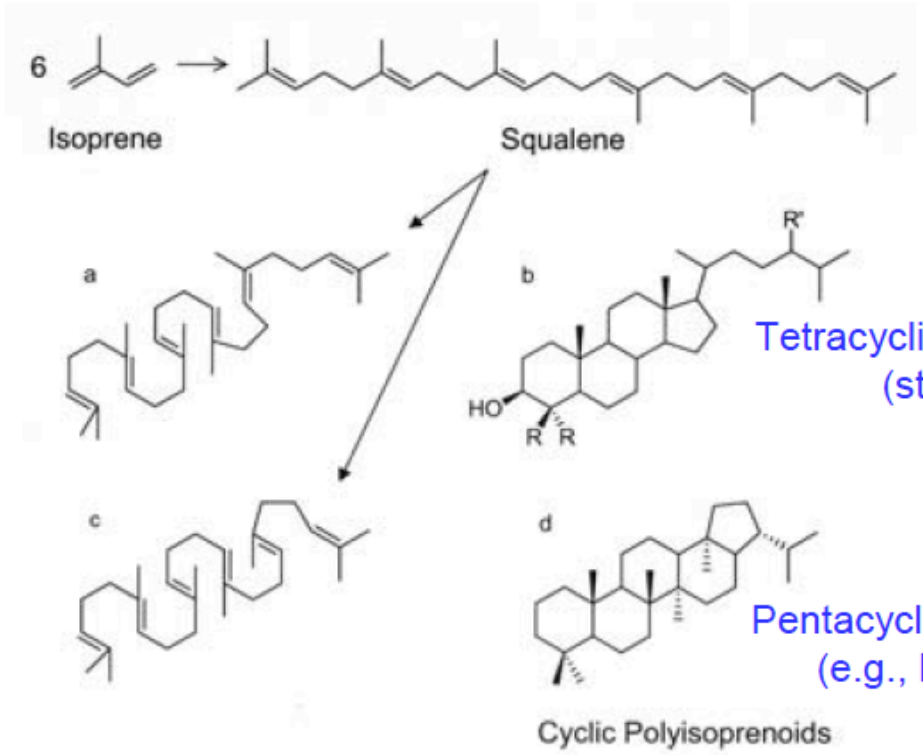
Batch cultures

Pathways of isoprenoid synthesis: Isotopic implications



*aka Deoxyxylulose pathway

Synthesis of cyclic isoprenoids (triterpenoids)



Tetracyclic triterpenoids
(steroids)

Pentacyclic triterpenoids
(e.g., hopanoids)

Isotopic distribution within biomarkers

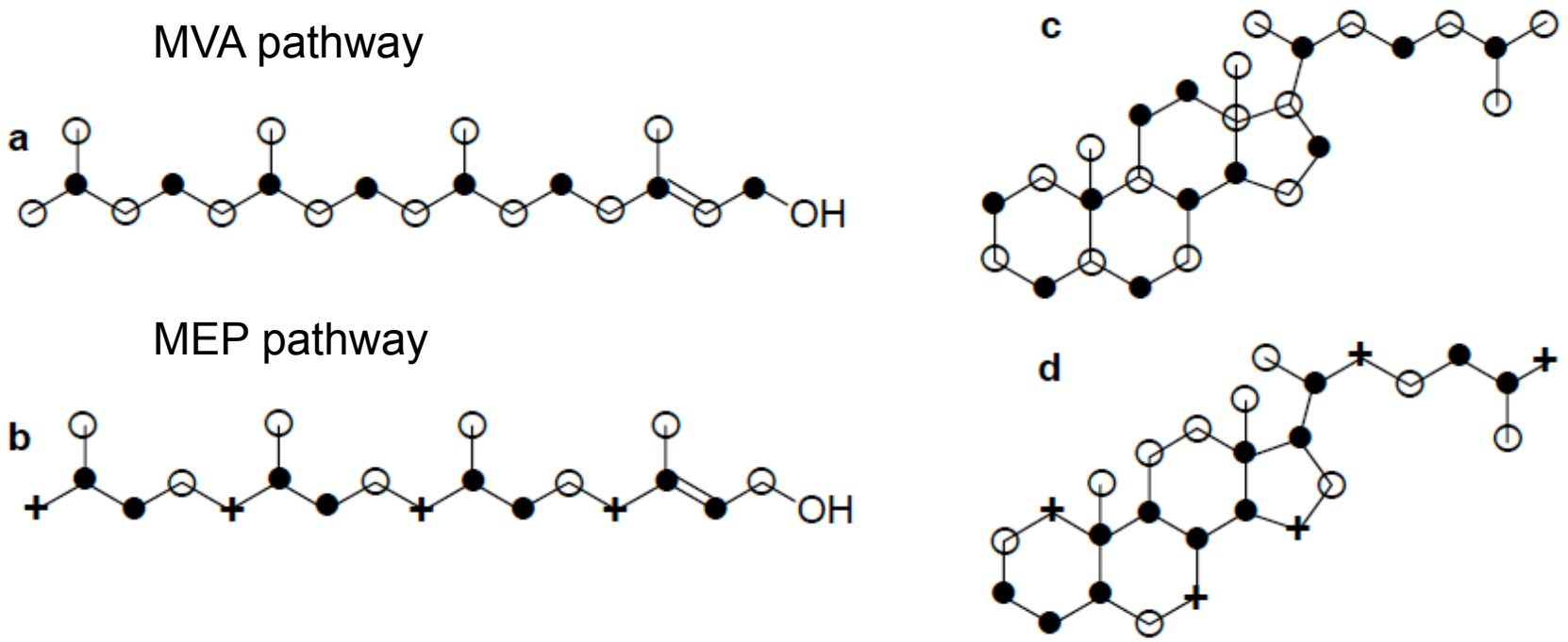


Figure 29. Relationships between the carbon positions in isopentenyl pyrophosphate and their sources. In the mevalonic-acid pathway, all five carbon positions in isopentenyl pyrophosphate derive from acetate and, in turn, from the C-1 + C-6 and C-2 + C5 positions of glucose. In the methyleryth-ritol-phosphate 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).