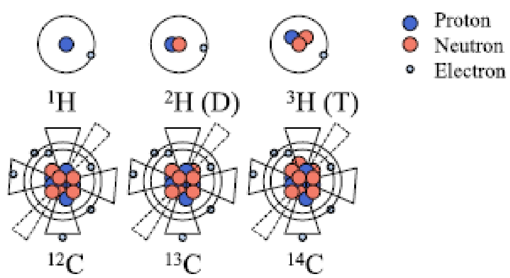
**Isotopes as tracers of biogeochemical processes**

Scott Saleska, 2/11/11

Outline

1. Isotope Definitions and terms
   a) Isotopes and isotope ratios.
   b) Kinetic fractionation; thermodynamic fractionation
   c) Simple illustration with the water cycle
2. CO2 isotopes in photosynthesis
   a) Photosynthetic discrimination in C3 plants
   b) C3 vs C4 photosynthesis and the distinction in isotopes
   c) Measuring isotopic composition of a flux
      i. Flux composition is not the same as concentration composition
      ii. Keeling plots

- Isotopes are atoms that contain the same number of protons but differ in the number of neutrons.



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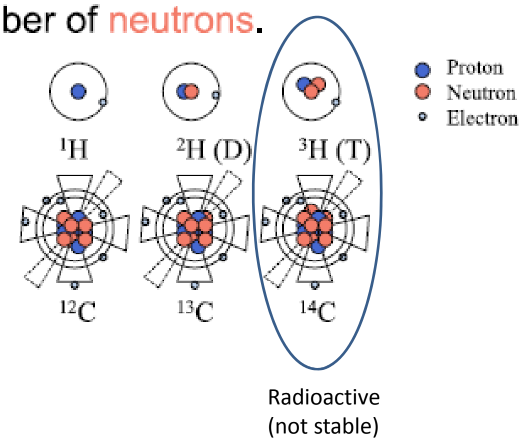



- Generally, isotope ratios are reported as the ratio of a heavy (rare) isotope to a light (primary) isotope:
  - $^{13}R_{CO2} = {}^{13}CO_2/{}^{12}CO_2$
  - $^{15}R_{N2} = {}^{15}N_2/{}^{14}N_2$

- Isotopic ratios expressed relative to a standard using delta notation: $\delta = [R_{sample}/R_{std} - 1]$

- $R_{sample}$ is the isotopic ratio of a sample (e.g., $^{18}R_{sample} = {}^{18}O/{}^{16}O_{sample}$)
- $R_{std}$ is the isotopic ratio of the standard, or reference material
- $R_{sample} < R_{std}$ gives negative $\delta$ value and is said to be *depleted*
- $R_{sample} > R_{std}$ gives positive $\delta$ value, said to be *enriched*

Example: For a sample with $^{18}O/^{16}O$=0.00198

$\delta^{18}O$ = [0.00198/0.00200 -1] = -0.010

Delta values are often expressed in units of permil (‰) by multiplying by a factor of 1000,

In this example, $\delta^{18}O$ is –0.010 (unitless) or –10‰

## Nominal abundances and standards

| Isotope Ratio | Nominal Abundance | Standard (form) | Accepted Standard Ratio* | Species Analyzed |
|---|---|---|---|---|
| $^{2}H/^{1}H$ (D/H) | 150ppm | SMOW (water) | 0.00015576 | $H_2$ |
| $^{13}C/^{12}C$ | 1.1% | PDB (carbonate) | 0.011224 | $CO_2$ |
| $^{17}O/^{16}O$ | 385ppm | SMOW (water) | 0.0003799 | $O_2$ |
| $^{18}O/^{16}O$ | 0.20% | SMOW (water) | 0.0020052 | $CO_2$ |
| $^{15}N/^{14}N$ | 0.37% | AIR ($N_2$ gas) | 0.003663 | $N_2$ |
| $^{34}S/^{32}S$ | 4.5% | CDT (FeS metal) | 0.04416 | $SO_2$ |
| $^{37}Cl/^{35}Cl$ | 32% | SMOC (chloride salt) | 0.3196 | $CH_3Cl$ |

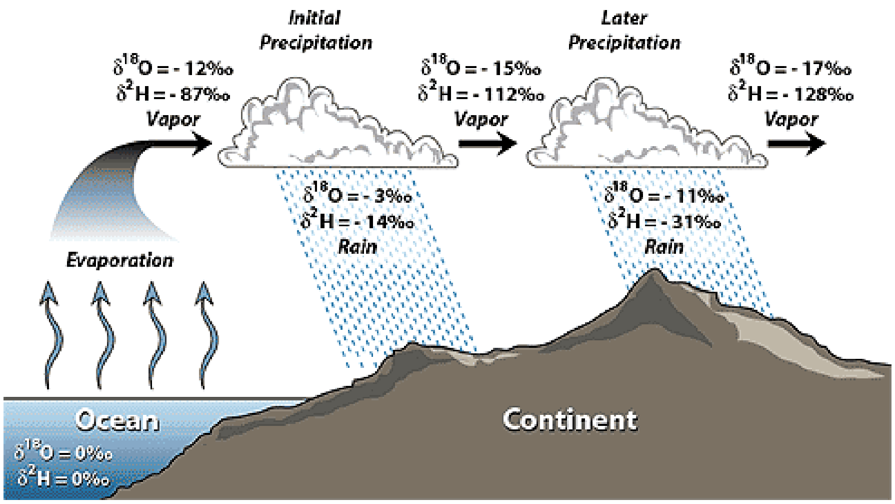
*Standards are certified by the International Atomic Energy Agency (IAEA). For more information, see Gröning, M., *in*: de Groot, P. A. (Ed.), Handbook of Stable Isotope Analytical Techniques, Elsevier, Amsterdam 2004, pp. 874–906.

## Two types of isotopic fractionation that cause changes in isotopic ratios

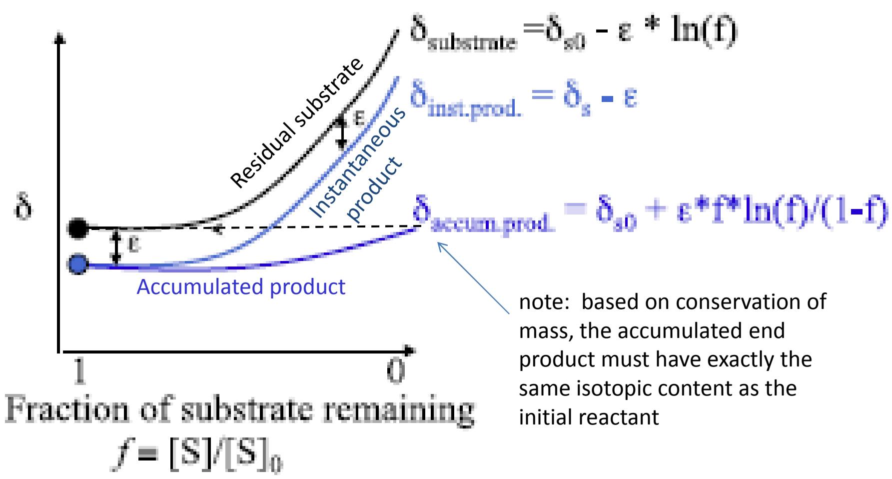
- *Kinetic isotope fractionation*:
  - One isotope reacts, diffuses, or evaporates faster than the other.
  - Can be due to chemical, physical, or biological processes.
  - Usually, the lighter isotope reacts or diffuses faster.
  - Magnitude of isotope effect is temperature, reaction rate, and species dependent
- *Equilibrium isotope fractionation*:
  - Exchange reactions in which a single atom is exchanged between 2 species (with isotopic preference).
  - Bidirectional (reversible) chemical reactions
  - Temperature dependent

## Simple Example – Rainfall

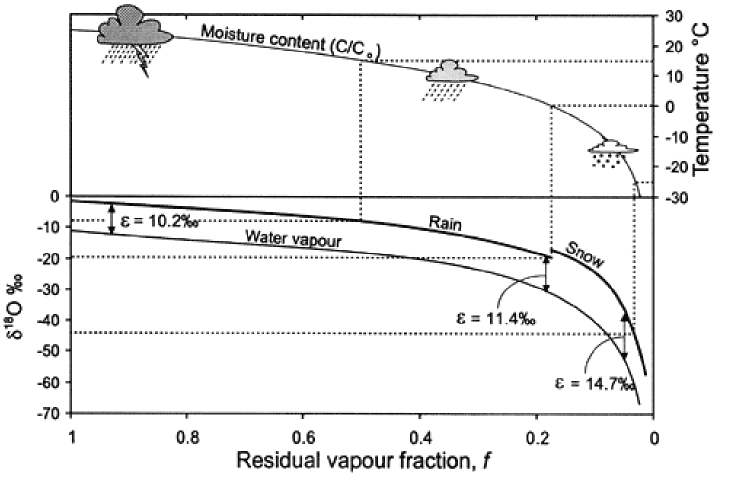
(note: evaporation prefers the lighter isotope, condensation the heavier)




In a closed system, isotope fractionation generates a <u>Raleigh fractionation curve</u>

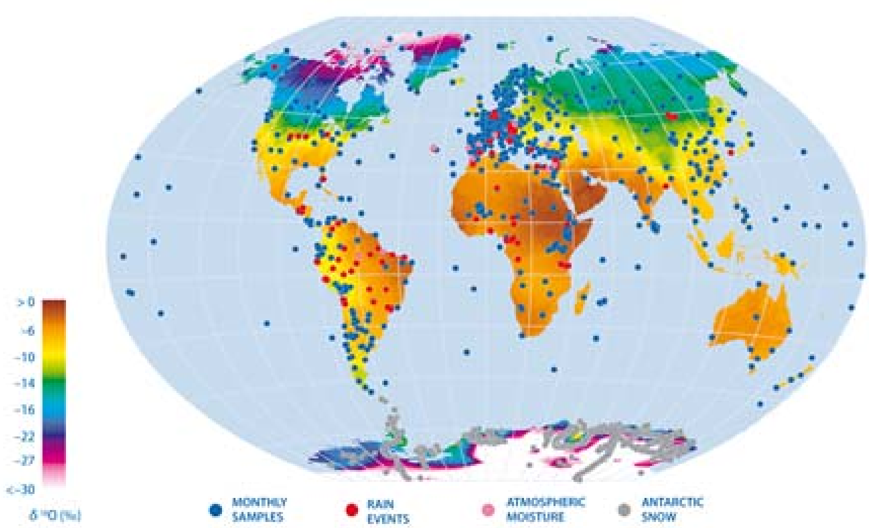


Raleigh fractionation/distillation curve for water vapor, with changing temperature drops to maintain saturation as vapor content of atmosphere drops



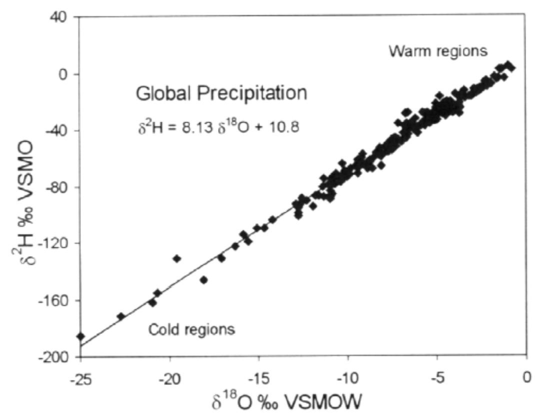

Note: direction of curve here is opposite (trending down) from previous slide because condensation prefers heavy isotope

# Global d18O




IAEA

## Global Meteoric Water Line (GMWL)

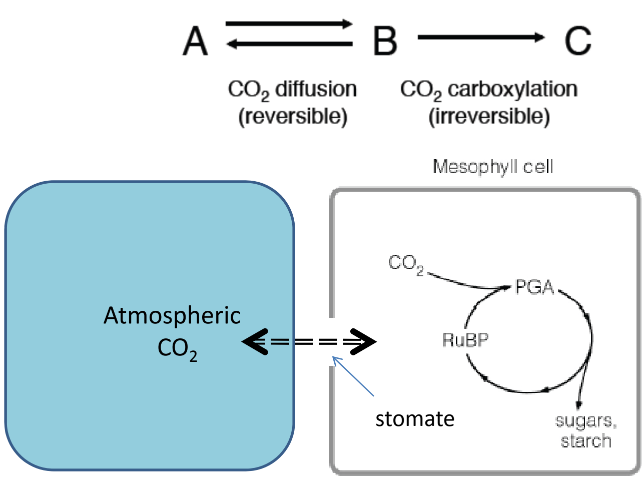



## Other examples from Biogeochemistry

| Substrate | | Product |
|---|---|---|
| $CO_2$ | photosynthesis $\varepsilon \approx 20‰$ | $C_{org}$ |
| $\delta^{13}C_{CO2}$ (-8 to -10‰) | | $\delta^{13}C_{org}$ (-18 to -23‰) |
| $NO_3^-$ | nitrate assimilation $\varepsilon \approx 5\text{-}10‰$ | $N_{org}$ |
| $\delta^{15}N_{NO3}$ (+5‰) | | $\delta^{15}N_{org}$ (-1 to +5 ‰) |
| $SO_4^{-2}$ | sulfate reduction $\varepsilon \approx 2\text{-}42‰$ | $S^{-2}$ |
| $\delta^{34}S_{SO4}$ (+20‰) | | $\delta^{34}S_{FeS2}$ (-45‰ to +20 ‰) |
| **$CH_3COOH$** | Methanogenesis **$\varepsilon_C$ = 25 - 35‰** | **$CO_2 + CH_4$** |
| **$\delta^{13}C_{acetate}$ (-28‰)** | | **$\delta^{13}C_{CH4}$(-50-60‰)** |

# Isotope discrimination by photosynthesis

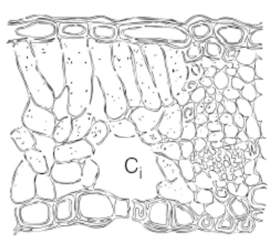
**Carbon isotope discrimination during photosynthesis is a two-stage process**



# Isotope effects (for $^{13}C/^{12}C$) in steps leading to $CO_2$ fixation in plants

| Process | Isotope Effect (α) | Discrimination (‰) | Symbol | Reference |
|---|---|---|---|---|
| diffusion of $CO_2$ in air through the stomatal pore | 1.0044 | 4.4 | $a$ | Craig |
| diffusion of $CO_2$ in air through the boundary layer to the stomatal | 1.0029 | 2.9 | $a_b$ | Farquhar |
| diffusion of dissolved $CO_2$ through $H_2O$ | 1.0007 | 0.7 | $a_l$ | O'Leary |
| net C3 fixation with respect to ci/ca | 1.027 | 27 | $b$ | Farquhar and Richards |
| fixation of gaseous $CO_2$ by Rubisco from higher plants | 1.030 (pH=8)<br>1.029 (pH=8.5) | 30<br>29 | $b_3$<br>$b_3$ | Roeske and O'Leary<br>Guy et al |
| fixation of $HCO_3^-$ by PEP carboxylase | 1.0020<br>1.0020 | 2.0<br>2.0 | $b_4^*$ | O'Leary et al<br>Reibach and Benedict |
| fixation of gaseous $CO_2$ (in equilibrium with $HCO_3^-$ at 25 °C) by PEP carboxylase | 0.9943 | -5.7 | $b_4$ | Farquhar |
| equilibrium hydration of $CO_2$ at 25 °C | 0.991<br>0.991 | -9.0<br>-9.0 | $e_b$ | Emrich et al<br>Mook et al |
| equilibrium dissolution of $CO_2$ into water | 1.0011<br>1.0011 | 1.1<br>1.1 | $e_s$ | Mook et al<br>O'Leary |

# Carbon isotope discrimination in $C_3$ plants




$C_a$

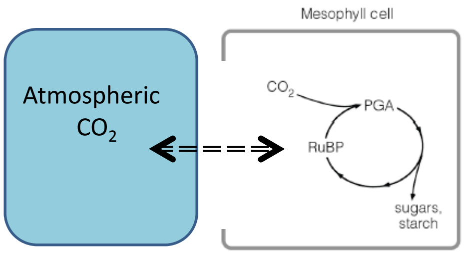
$$\Delta = a\frac{c_a - c_i}{c_a} + b\frac{c_i}{c_a} = a + (b - a)\frac{c_i}{c_a}$$

4.4 — diffusion

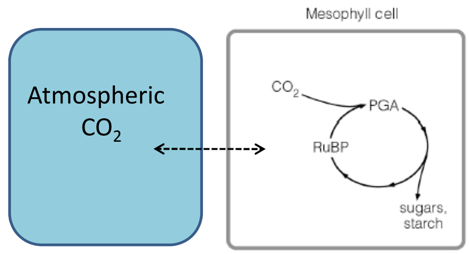
27 — Net biochemical fixation

Stomatal 'openness' determines expression of biochemical fractionation

# Consider two extremes




Stomates open to atm ($c_i \approx c_a$) :
**$\Delta = a + (b - a) * 1 = b = 27‰$**




Stomates closed ($c_i \approx 0$) :
**$\Delta = a + (b - a) * 0 = a = 4.4‰$**

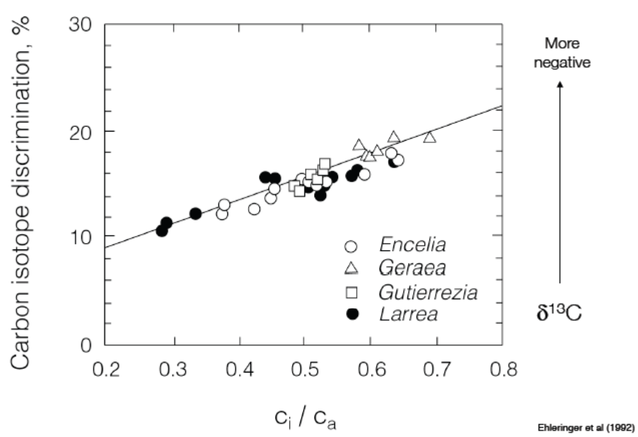
$$\Delta = a\frac{c_a - c_i}{c_a} + b\frac{c_i}{c_a} = a + (b - a)\frac{c_i}{c_a}$$

4.4 — diffusion

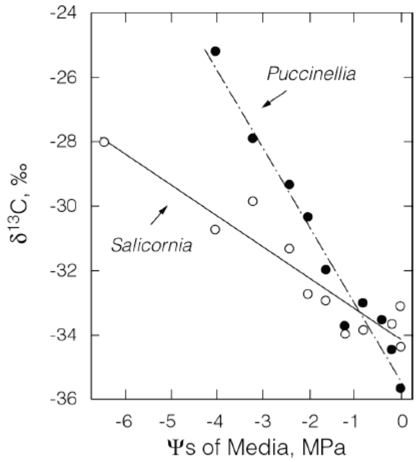
27 — Net biochemical fixation

Stomatal 'openness' determines expression of biochemical fractionation

## $C_3$ carbon isotope discrimination follows predicted relationship

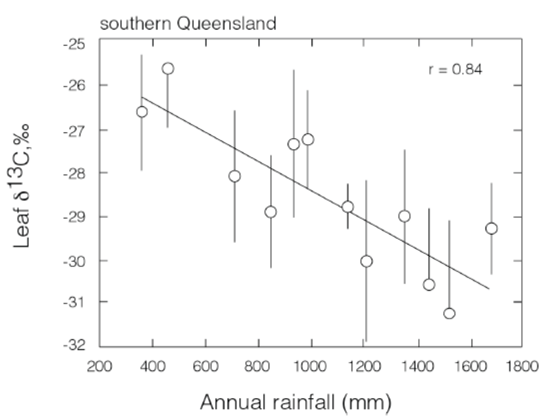


## $C_3$ plants discriminate less when exposed to water stress




Guy et al. (1980)

## There is a decrease in $C_3$ discrimination along aridity gradients




Stewart et al. (1995)

# Photosynthetic Pathway Variation

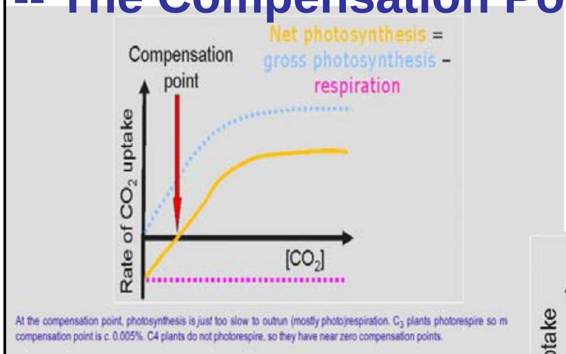
- **C3** – $CO_2$ fixation by Rubisco; PGA (phosphoglycerate – a 3 carbon sugar) is the product of initial carboxylation
- **C4** – $CO_2$ fixation by PEP carboxylase to produce OAA (oxaloacetate – a 4 carbon acid); C4 then transported within the leaf, decarboxylation and re-fixation by Rubisco.
- **CAM** (crassulacean acid metabolism) – $CO_2$ fixation by PEP carboxylase to OAA, but results in the production of malic acid in the vacuole during the day and de-carboxylation and fixation at night

# Why C4 plants?

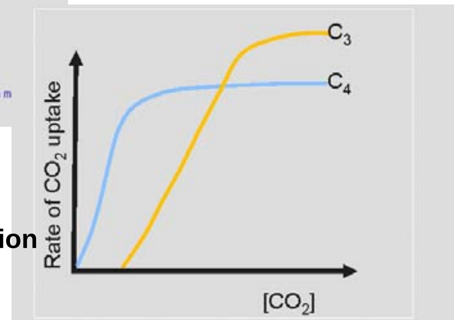
## Problems with C3 photosynthesis

Increase in photorespiration - in hot dry conditions
- C3 plants conserve water by closing stomates, decreasing intercellular [CO2]
- Competition between O2 and CO2 for Rubisco binding site
- Photorespiration increases when $[O_2]$ / $[CO_2]$ inside the leaf increases

## Why C4 plants? CO2 limitation -- The Compensation Point



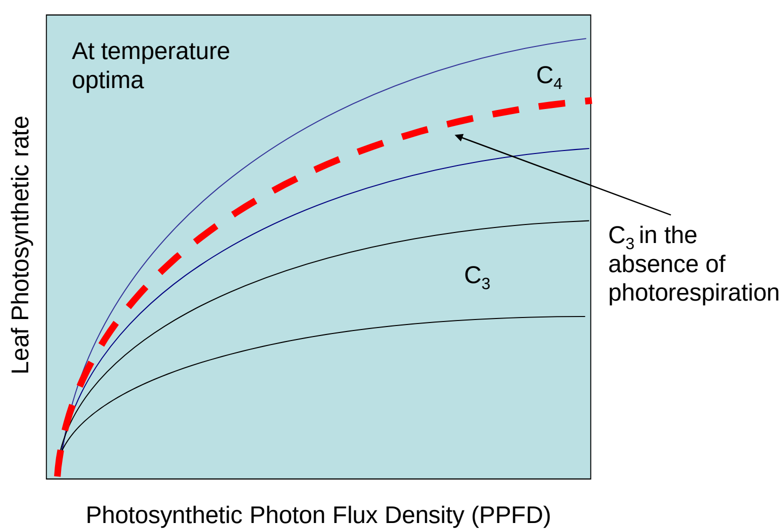

**Compensation points for C3 and C4 differ**



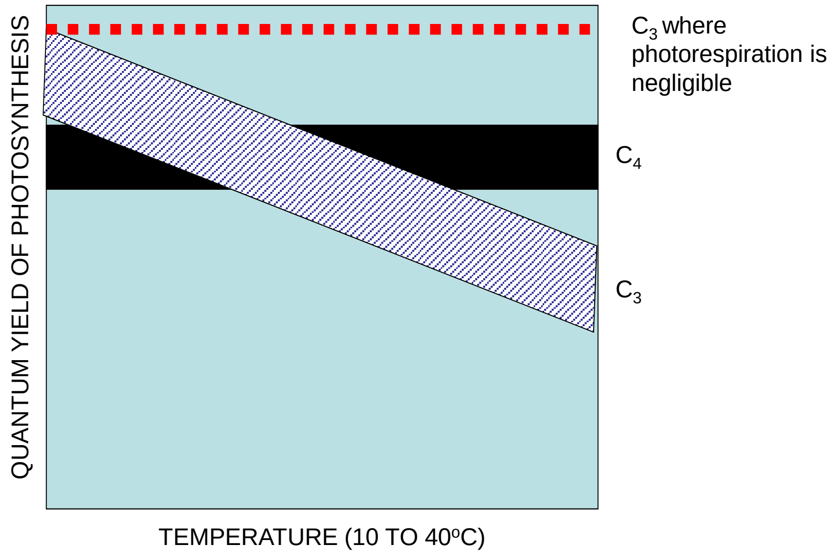

**Net Assimilation =**
**gross photosynthesis - respiration**

http://www.steve.gb.com/science/photosynthesis_and_respiration.html

## Light-use efficiency

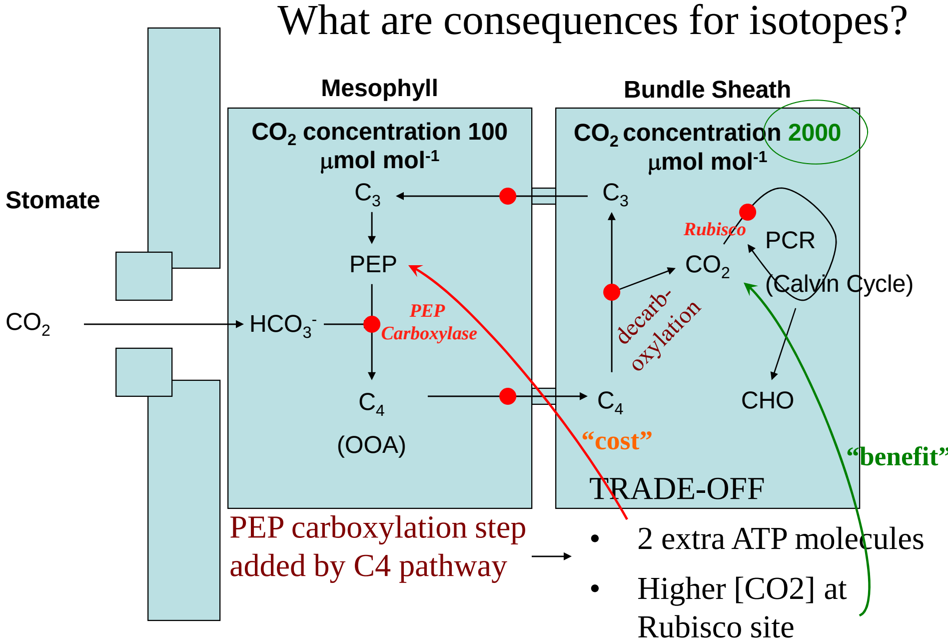


## Light-use efficiency




## C4 Photosynthesis: a 2-step biochemical process

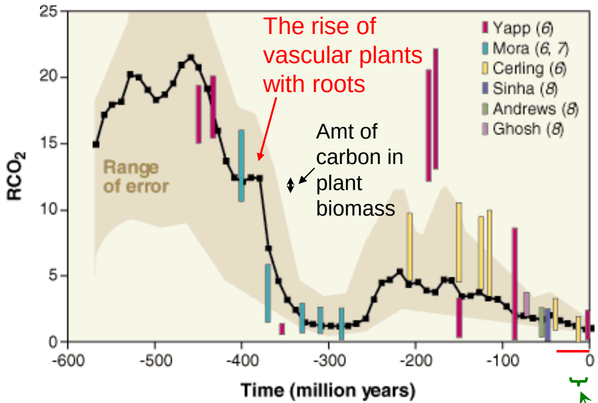
What are consequences for isotopes?




- 2 extra ATP molecules
- Higher [CO2] at Rubisco site

# Long-term trends in atmospheric CO2

Ratio of ancient to modern pre-industrial CO2

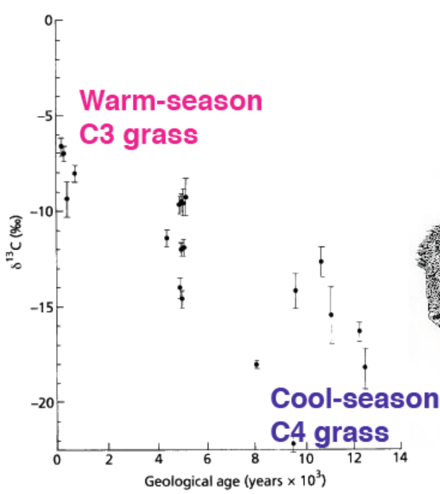


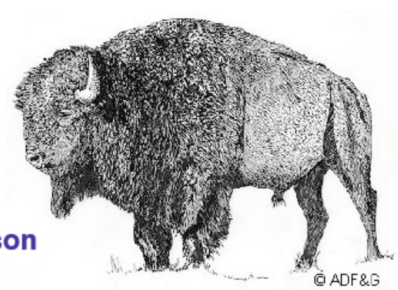

Continuous downward trend over last 50 Ma

Evolution of C4 plants

Berner, 1997

## You are what you eat (isotopically)
## Example from fossil bison



## You are what you eat (isotopically)
## Example from Beer

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. 2002, 50, 6413–6418 6413

**Heavy and Light Beer: A Carbon Isotope Approach To Detect $C_4$ Carbon in Beers of Different Origins, Styles, and Prices**

J. Renée Brooks,*,† Nina Buchmann,‡ Sue Phillips,†,+ Bruce Ehleringer,§ R. David Evans,⊥ Mike Lott,† Luiz A. Martinelli,‖ William T. Pockman,# Darren Sandquist,⊗ Jed P. Sparks,∇ Lynda Sperry,× Dave Williams,† and James R. Ehleringer†

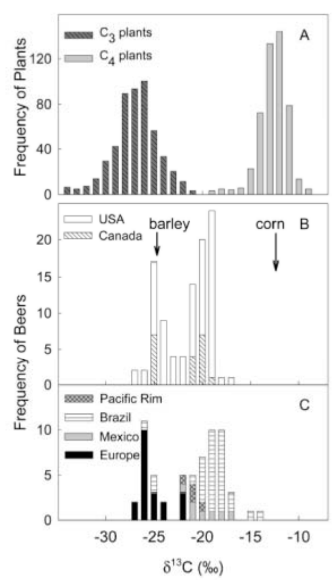

## You are what you eat (isotopically)

**Reinheitsgebot**

**1516 German beer purity law:**
"Furthermore, we wish to emphasize that in future in all cities, markets and in the country, the only ingredients used for the brewing of beer must be **Barley**, **Hops** and **Water**. Whosoever knowingly disregards or transgresses upon this ordinance, shall be punished by the Court authorities' confiscating such barrels of beer, without fail."

**Adjuncts** - nonessential ingredients

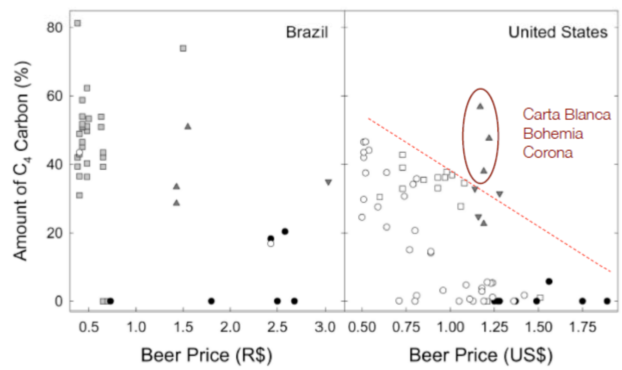
**Corn sugar** and **corn starch** (C4) are used as adjuncts to reduce manufacturing costs and increase alcohol content




Brooks et al (2002)

# You are what you eat (isotopically)

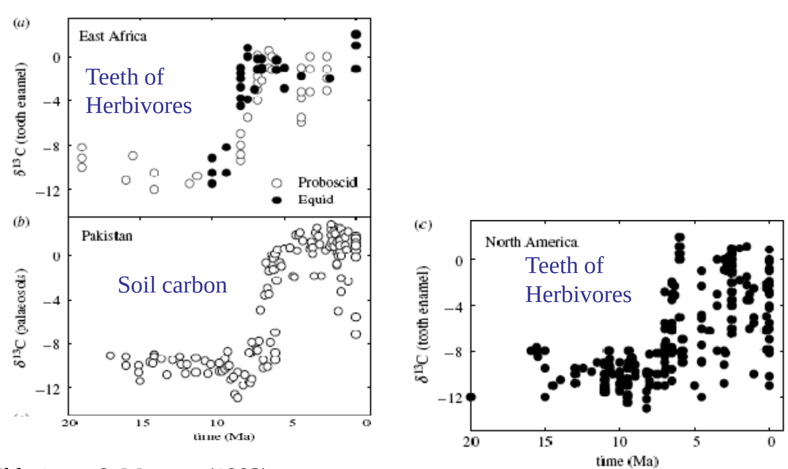
## You get what you pay for!




Brooks et al (2002)

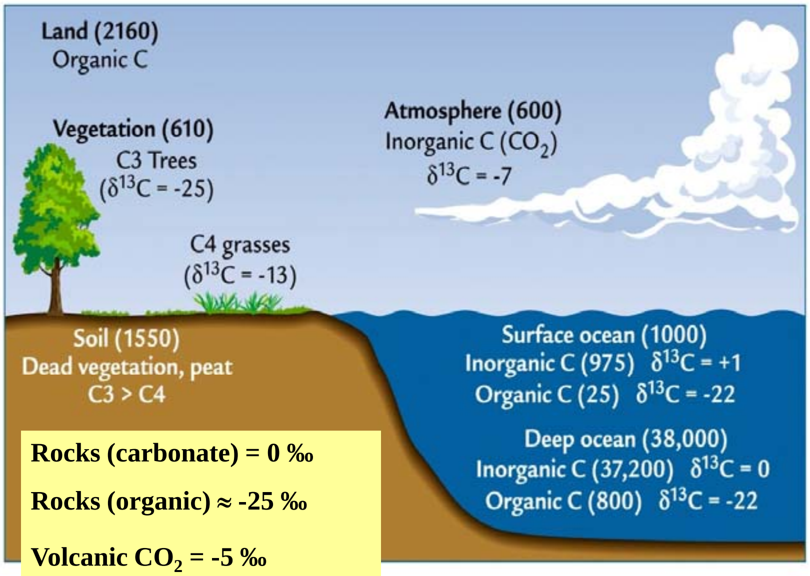
Apparent synchronicity in rise in C4 at sites around the world.

Paleo-Indicators of C4 plant prevalence

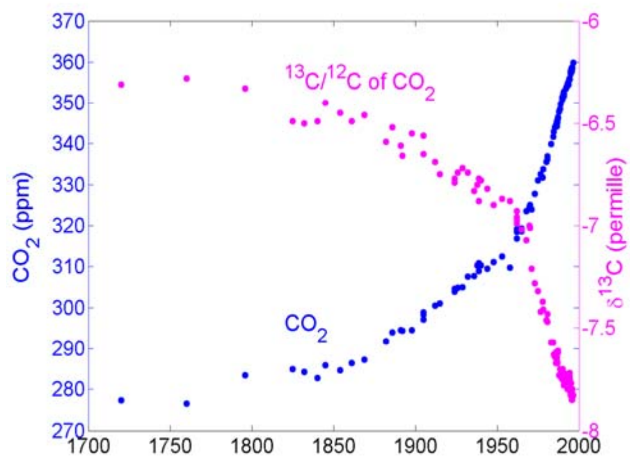



Ehleringer & Monson (1993)

## Size and $\delta^{13}C$ (vs PDB) of Earth's Carbon Reservoirs




## Atmospheric "$^{13}C$ Suess Effect"




[Francey et al., 1999]