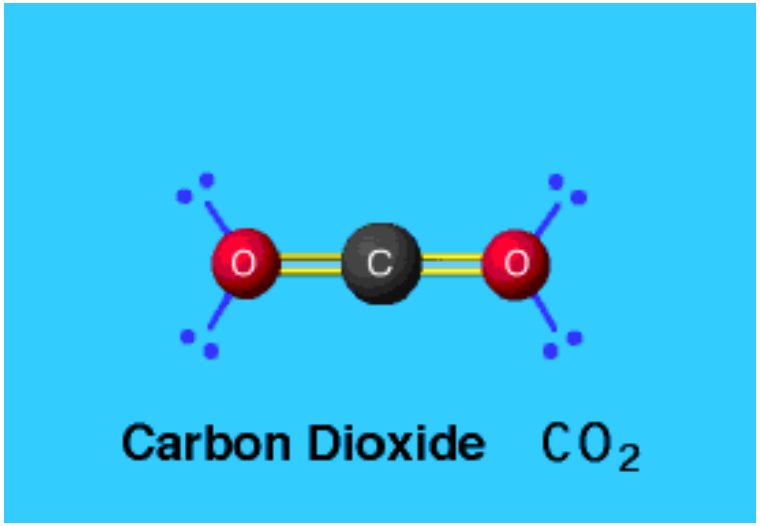
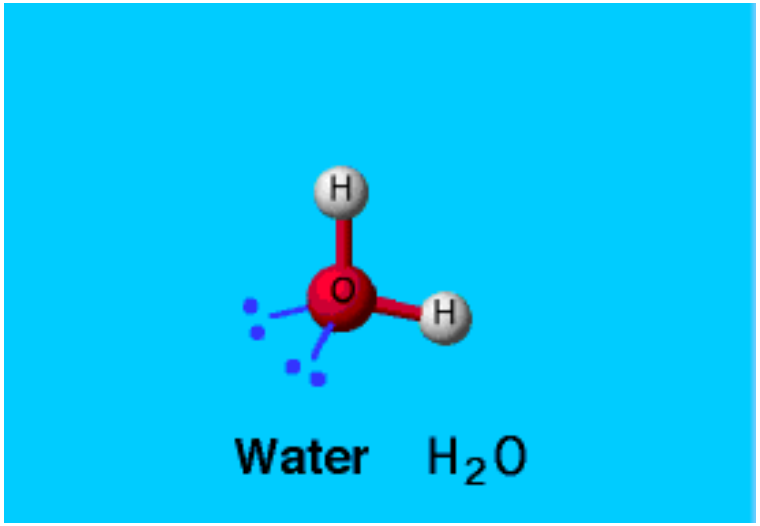
Stable Isotopes in Ecosystem Ecology

- **Objectives**
  - To introduce:
    - Basic terminology, definitions, formulas, etc.
    - C, H, O, and N stable isotopes and their application in ecology





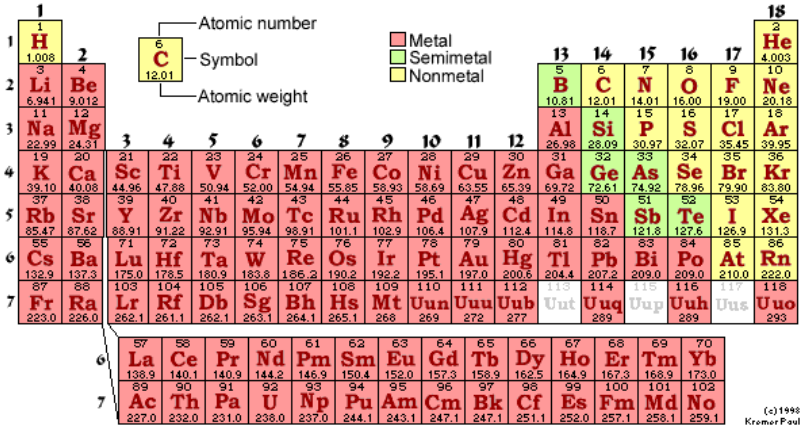
Stable Isotopes in Ecosystem Ecology

- Useful for
  - Identifying source materials
  - Quantifying fates (natural tracers)
  - Inferring processes that cannot be directly quantified
  - Estimating rates that cannot be directly quantified
  - Widely used in ecological studies today
    - C cycling, H_2O source, N dynamics, etc.

What are isotopes, and where do they come from?

Periodic Table of the Elements

- **Crash course in chemistry**
  - Atomic Number = # of protons
    - Unique to that element; never changes
    - ~Neutral charge, so same # of electrons
      - # of electrons determines the chemical properties of an atom
  - Mass Number = # of protons + neutrons
    - Round atomic weight to nearest whole #
    - Atomic Weight is weighted average of all naturally occurring isotopes
  - # of neutrons = Mass number - atomic number
    - How many neutrons does C have?
    - Krypton?



Origin of Atoms / Elements

- **The BIG BANG initiated the fusion of "quarks" to form protons (^{1}H) and neutrons**
  - **Fusion of protons and neutrons → hydrogen (^{1}H) & helium (^{4}He)**

- **Stars formed ~1 billion years later**
- **As a star ages → collapses inward & temperature ↑**
  - **^{4}He is "burned" and new elements are synthesized**

    ^{1}H + protons and neutrons <----> ^{4}He

    $^{4}He + {}^{4}He \leftrightarrow {}^{8}Be$

    $^{8}Be + {}^{4}He \rightarrow {}^{12}C$

    $^{12}C + {}^{4}He \rightarrow {}^{16}O$

Origin of Atoms / Elements

- Further burning reactions synthesize more new elements

  $^{12}C + {}^{12}C \rightarrow {}^{24}Mg$

  $^{16}O + {}^{16}O \rightarrow {}^{32}S$

- Loss of ^{4}He in larger elements creates smaller elements

  $^{24}Mg - {}^{4}He \rightarrow {}^{20}Ne$

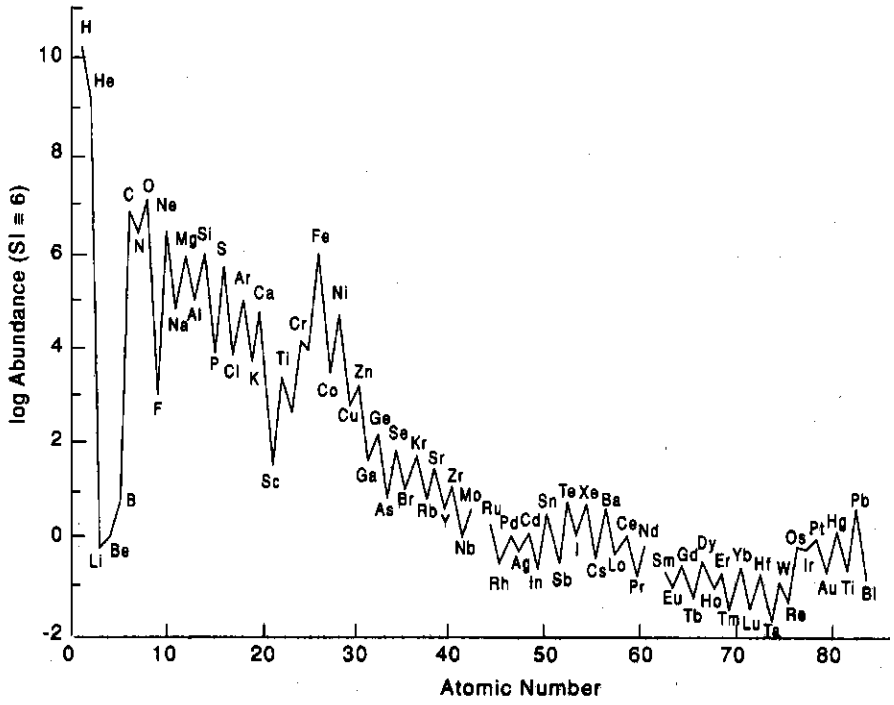
  $^{32}S - {}^{4}He \rightarrow {}^{28}Si$

- Elements with atomic # >30 form when Fe captures neutrons during supernovas
- Fission and radioactive decay account for odd-numbered elements, and isotopes

  $^{16}O + {}^{16}O \rightarrow {}^{32}S \rightarrow {}^{31}P + {}^{1}H$

Origin of Atoms / Elements

- **Cosmic Abundance of elements in the Universe**
  - Light elements (Atomic # < 30) far more abundant than heavier elements
    - 3 exceptions
  - Even-numbered elements more abundant than odd-numbered elements
    - Esp. among lighter elements



Schlesinger 1997

Stable Isotopes

- Composition of an atom ("nuclide") is described by the number of protons and neutrons in the nucleus:

  $A = Z + N$

  where A is mass number (atomic weight rounded to nearest whole number), Z is number of protons (=atomic number), N is number of neutrons

  - ^{12}C has 6 protons and 6 neutrons (mass number = 12; atomic number (# of protons) = 6)

Stable Isotopes

- **Isotopes (nuclides) are forms of the same element that differ in the number of neutrons in the nucleus**
  - **Formed from nucleosynthesis (Big Bang; star formation/collapse), and for radiogenic isotopes from decay**
  - **Stable isotopes are those that do not decay over time**
    - **vs. radioactive isotopes**
  - **# of protons (and, therefore, electrons) is the same, so isotopes have only subtle chemical differences**
  - **However, isotopes differ in mass**
  - **C has 6 protons, so ^{12}C has 6 neutrons, and ^{13}C has 7 neutrons, and ^{14}C has 8 neutrons**
    - **Atomic weights differ (12, 13, and 14)**
    - **Why is C listed in periodic tables as having an atomic weight of 12.01?**
    - **What does that tell you about the ratio of C isotopes?**

Stable Isotopes

- H, C, N, and O most relevant for ecological applications
  - Why?
  - P?
- Lighter isotope is always more abundant
  - Why?

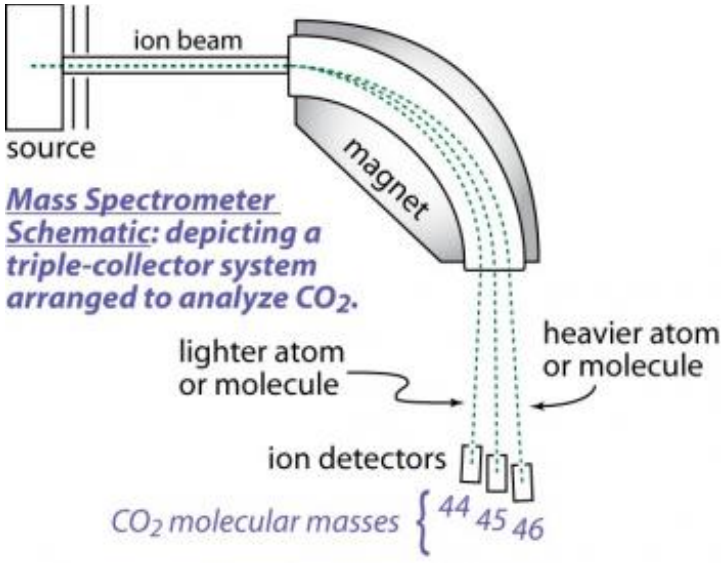
Table I. Average abundances of stable isotopes that are important for understanding ecological systems

Element	Isotope	Average abundance (%)
Hydrogen	^{1}H	99.985
	^{2}H	0.015
Carbon	^{12}C	98.89
	^{13}C	1.11
Nitrogen	^{14}N	99.63
	^{15}N	0.37
Oxygen	^{16}O	99.759
	^{17}O	0.037
	^{18}O	0.204
Sulfur	^{32}S	95.00
	^{33}S	0.76
	^{34}S	4.22
	^{35}S	0.014

West et al. 2006

Stable Isotopes - Terms and Definitions

- "Heavy" stable isotopes are rare in abundance but can be measured very accurately by isotope ratio mass spectrometers (IRMS)
- The ratio of **heavy : light isotopes (rare : abundant)**
  - ratio in a sample is compared with the ratio in a standard
  - ratio in two samples can also be compared



Stable Isotopes - Terms and Definitions

- **Delta (d) notation used to express stable isotope values:**

$$d^{13}C = ([(^{13}C/^{12}C)_{sample} / (^{13}C/^{12}C)_{standard}] - 1) * 1000$$

$$d^{13}C = ([R_{sample} / R_{standard}] - 1) * 1000$$

- **d values expressed in parts per thousand, or "per mil" (‰), for ease of interpretation**

- **International standards exist, so all delta values can be compared**
  - **PDB limestone for C, Standard Mean Ocean Water (SMOW) for H and O, atmospheric N_2 for N**

Stable Isotopes - Terms and Definitions

- **If a sample has more heavy isotope than the standard (or than another substance) it is "enriched" or "heavier", and if it has less heavy isotope it is "depleted" or "lighter"**

$$\delta^{13}C = ([R_{sample} / R_{standard}] - 1) * 1000$$

(where R = $^{13}C/^{12}C$)

Stable Isotopes - Terms and Definitions

- **The more negative a δ value is, the smaller the amount of heavy isotope → more depleted**
- **The more positive a δ value is, the larger the amount of heavy isotope → more enriched**
- **Example: C ranges from -50‰ to +50‰:**

  $\delta^{13}C$ of PDB = 0‰

  $\delta^{13}C$ of atmospheric CO_2 = -8‰

  $\delta^{13}C$ of C_3 plants is around -28‰

Stable Isotopes - Terms and Definitions

- **Isotopic differences in different substances are a result of fractionation (α) that occurs in nature → produces different isotopic ratios in sources and products**
  - **Kinetic: unidirectional; due to differences in diffusion coefficients, enzymatic preference for one isotope over another**
  - **Equilibrium: bidirectional; between two substances in equilibrium (e.g., phase changes; vapor ↔ liquid)**
    - **Heavy isotope concentrates where it is most strongly bonded**
    - **Impacted by temperature - less fractionation at higher temperatures because reactions occur more rapidly**

Stable Isotopes - Terms and Definitions

- **Fractionation factor (a) typically expressed as <u>discrimination</u> (D; ‰)**
  - **How much the heavy isotope is fractionated (i.e., discriminated against) from source → product**
    - **Can only get discrimination if all of the source is not consumed**

$$\alpha = R_{source}/R_{product} \text{ (e.g, } R_{air}/R_{plant})$$

$$D = (\alpha - 1)*1000, \text{ or } (R_{source}/R_{product} - 1)*1000$$

$$D = (d_{source} - d_{product})/(1 + d_{product})$$

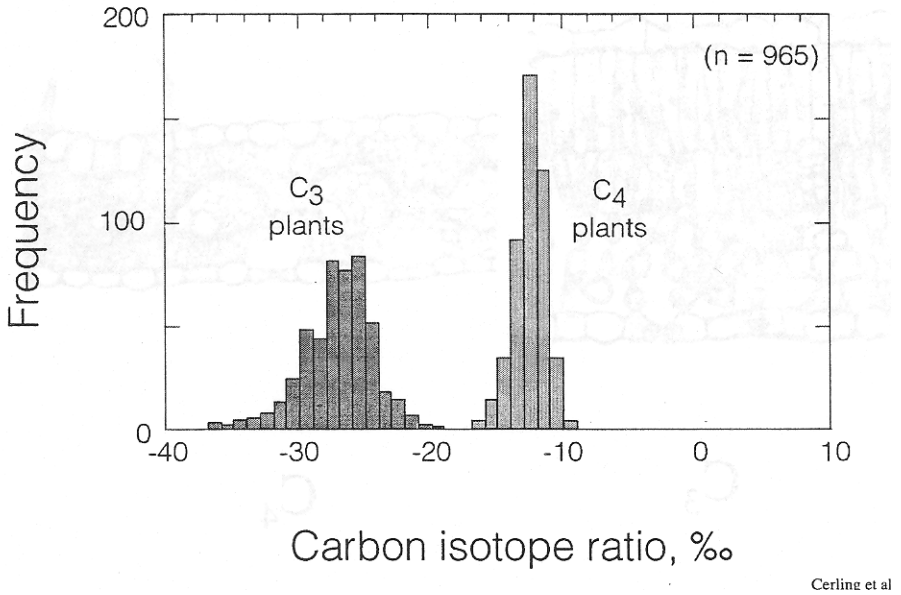
$$D \approx d_{source} - d_{product}$$

Photosynthesis and C isotopes

C_3 and C_4 plants have different C isotope ratios

C_3 ~ -28 and C_4 ~ -12

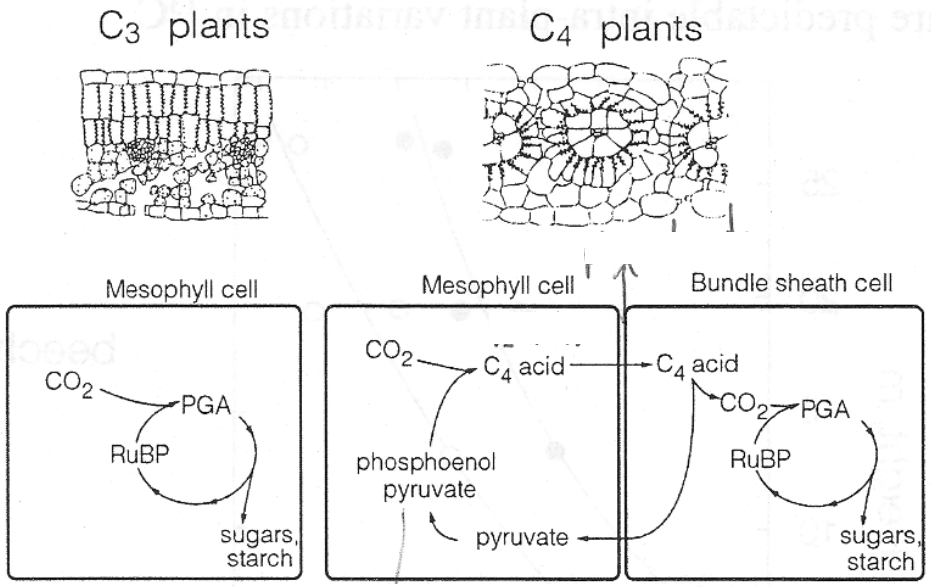
Why?



Cerling et al. 1997

Photosynthesis and C isotopes

C_3 and C_4 plants have different photosynthetic pathways



C_3 plants

- cool-season grasses
- most trees
- protein crops
- rice

C_4 plants

- warm-season grasses
- corn
- sorghum
- millet

Photosynthesis and C isotopes

- **Two main steps in which $^{13}CO_2$ is discriminated against: diffusion and carboxylation (kinetic effects)**

  - **Diffusion of heavy atoms or molecules is slower than diffusion of lighter ones**

  - **Enzymatic discrimination by Rubisco and PEP carboxylase discriminates (strongly) against ^{13}CO during carboxylation**

Photosynthesis and C isotopes

- **For C_3 plants** ($\delta \approx$ **-28‰**)
  - **Fractionation due to diffusion** ≈ 4.4‰ **and fractionation due to Rubisco** ≈ 27‰
  - **Also depends on** c_i/c_a

$$\Delta^{13}C_P = a + (b - a)^* c_i/c_a$$

Where a **is the fractionation associated with diffusion (4.4‰), and** b **is the fractionation associated with Rubisco (27‰)**

*$\Delta \approx 20$ (**-8‰ - -28‰ = +20‰**); **Actual range = 13-25‰**

* $c_i/c_a \approx 0.69$

Photosynthesis and C isotopes

- For C_4 plants, have to separate fractionation factors for PEP carboxlyase and Rubisco
  - PEP carboxylase fixes carbon in the mesophyll for transport into bundle sheath cells.
  - In the bundle sheath cells, Rubisco is physically isolated from the stomatal cavity.
    - In the bundle sheath cells all of the substrate is utilized, so there is no Rubisco fractionation…
      - Except CO_2 (or HCO_3^-) "leaks" out of bundle sheath cells back into the stomatal cavity

Photosynthesis and C isotopes

- For C_4 plants (d ≈ **-12‰**)

$$D^{13}C_P = a + (b_4 + b_3 f - a)^* c_i/c_a$$

Where ***a*** **is the fractionation associated with diffusion (4.4** ‰), b_4 is the fractionation associated with PEP carboxylase (-5.7‰), b_3 is the fractionation associated with Rubisco (27‰), and f is the leakiness of the bundle sheath

*D ≈ 4 (**-8‰ - -12 ‰) = +4‰); Actual range = 2.5-5‰**

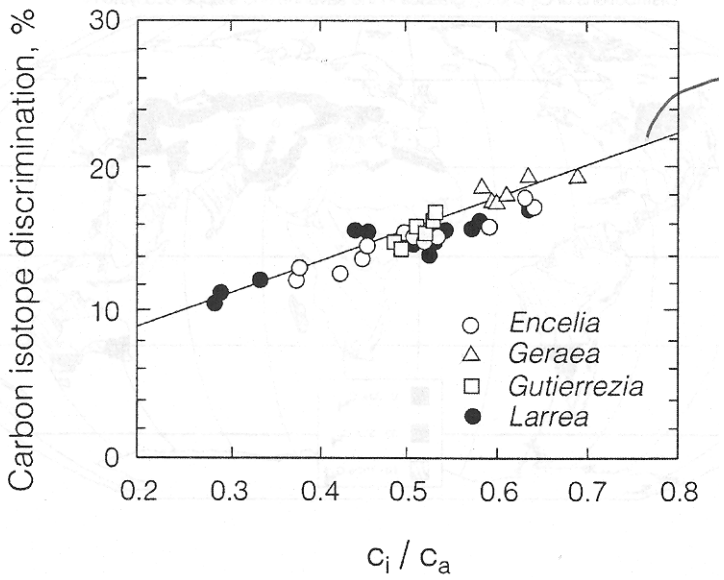
***(b_4 + b_3 f - a)** ≈ 0 in most C_4 **plants**

***c_i/c_a** **is trivial for most C_4 plants**

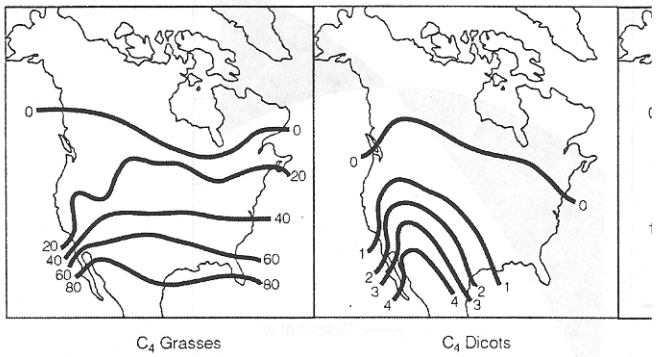
f ≈ 0.4 (leakiness plays an important role)

Photosynthesis and C isotopes

- c_i/c_a important for C_3 plants, not so much for C_4 plants
- C_4 plants more adapted to arid conditions



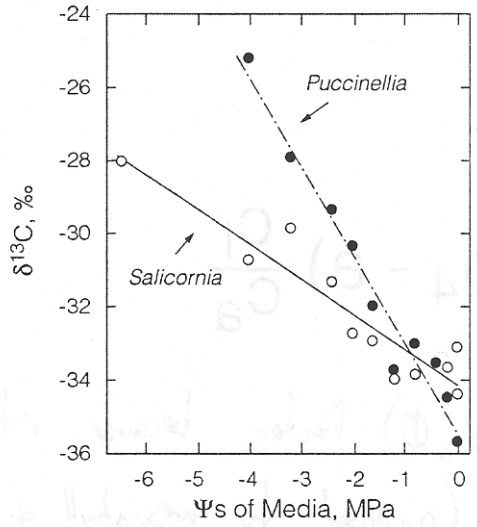
Ehleringer et al. 1992



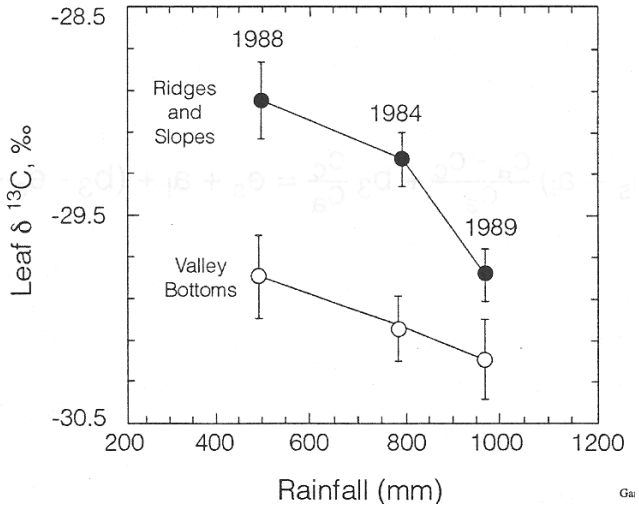
Ehleringer 1979

Photosynthesis and C isotopes

- C_3 plants discriminate less when exposed to water stress



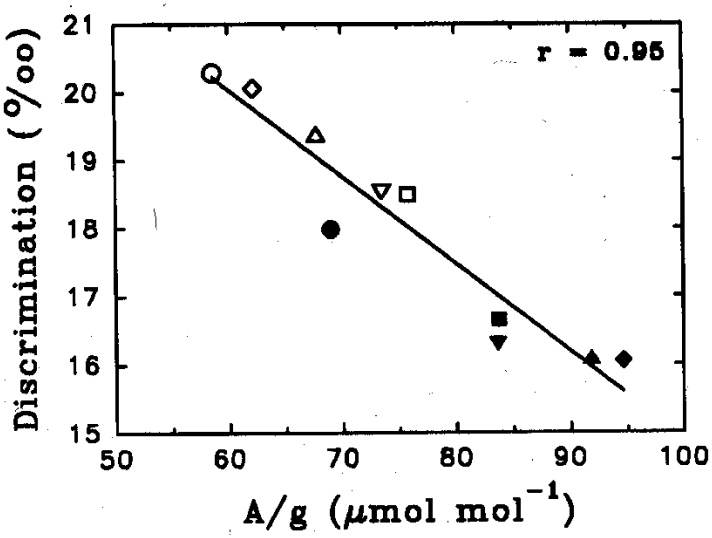
Guy et al. 1980



Garten & Taylor 1992

Utility of C isotopes

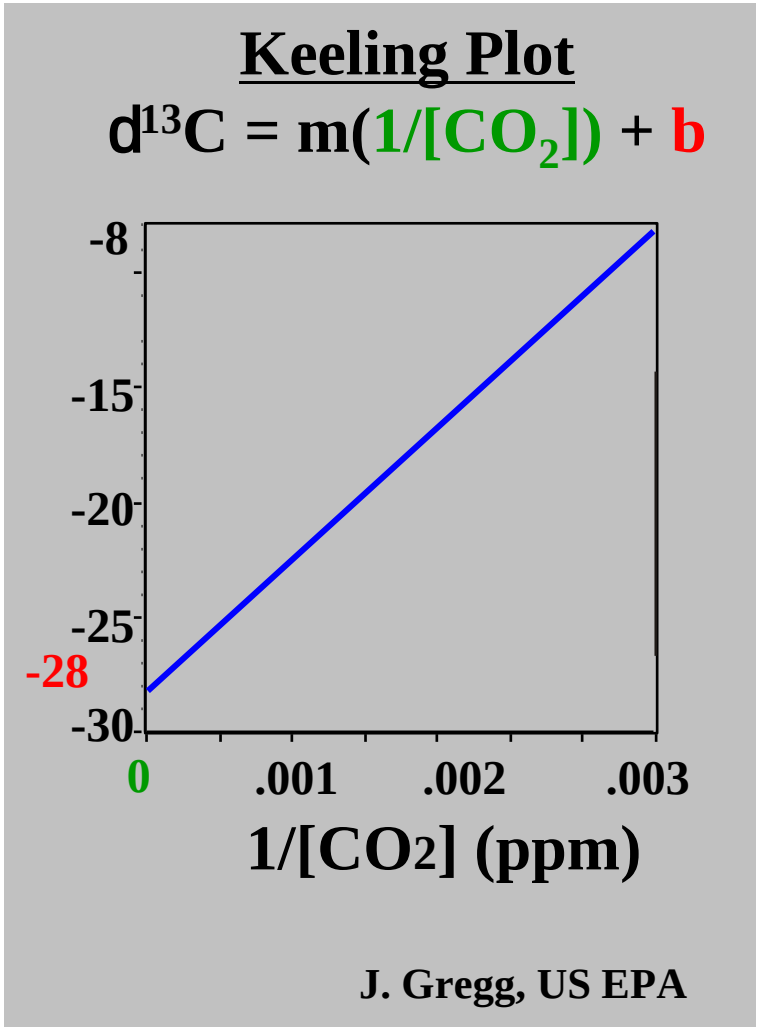
- Water use efficiency = C gain per unit of H_2O lost
- C uptake & discrimination, and water loss are all dependent on c_i/c_a
- $d^{13}C$ values in C_3 plant tissue are often considered to represent a time weighted average WUE



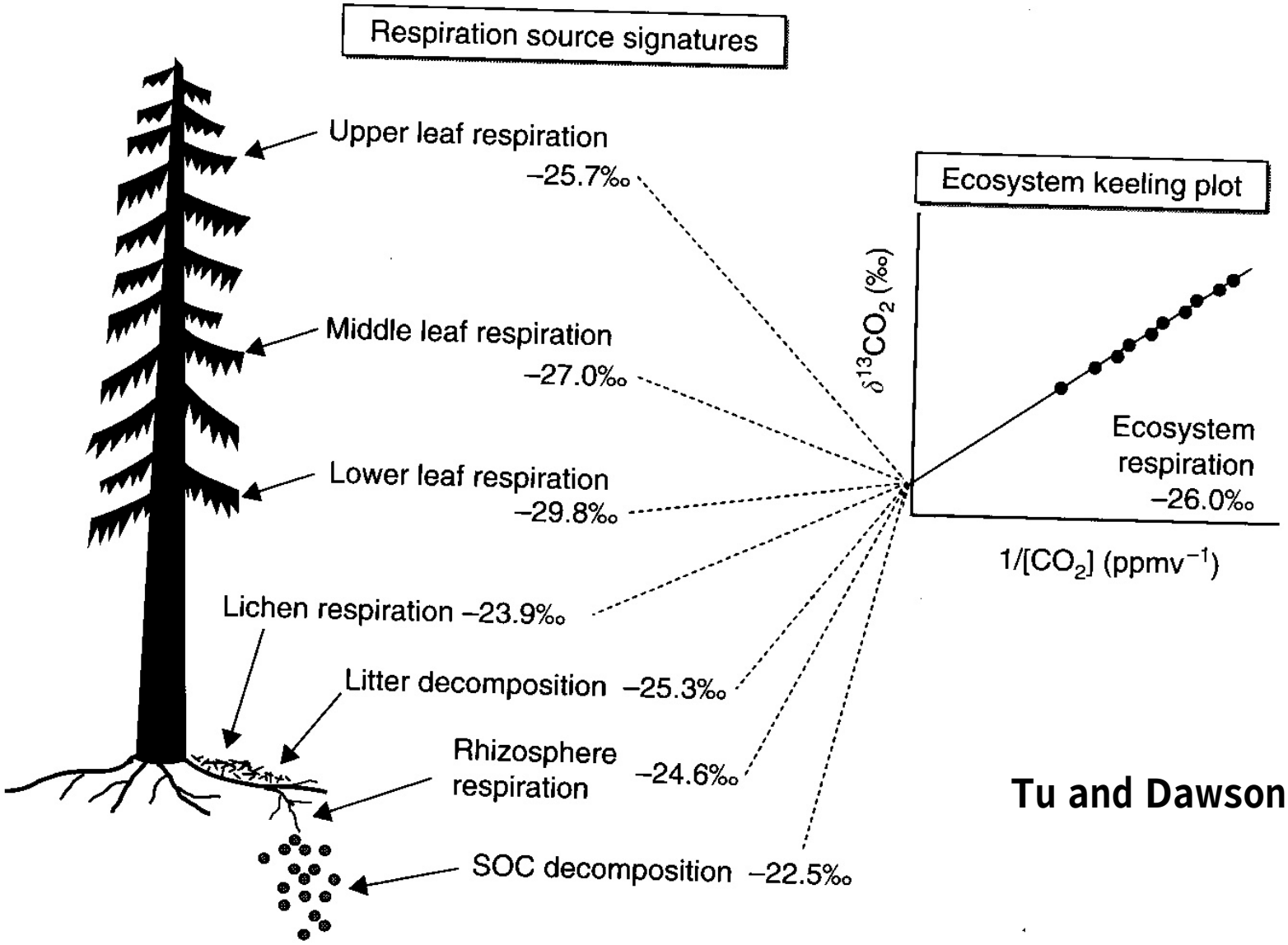
Cultivars under water stressed (closed symbols) and irrigated (open symbols) conditions (Meinzer et al., 1993).

Utility of C Isotopes

- **Keeling Plots can be used to trace sources of CO_2 flux**
  - **Multiple measurements of $\delta^{13}C$ over a range of CO_2 conc.**
    - **Linear regression model**
  - **Y-intercept of slope is the δ value of the source(s)**



Utility of C Isotopes



Tu and Dawson, 2005

Utility of C Isotopes

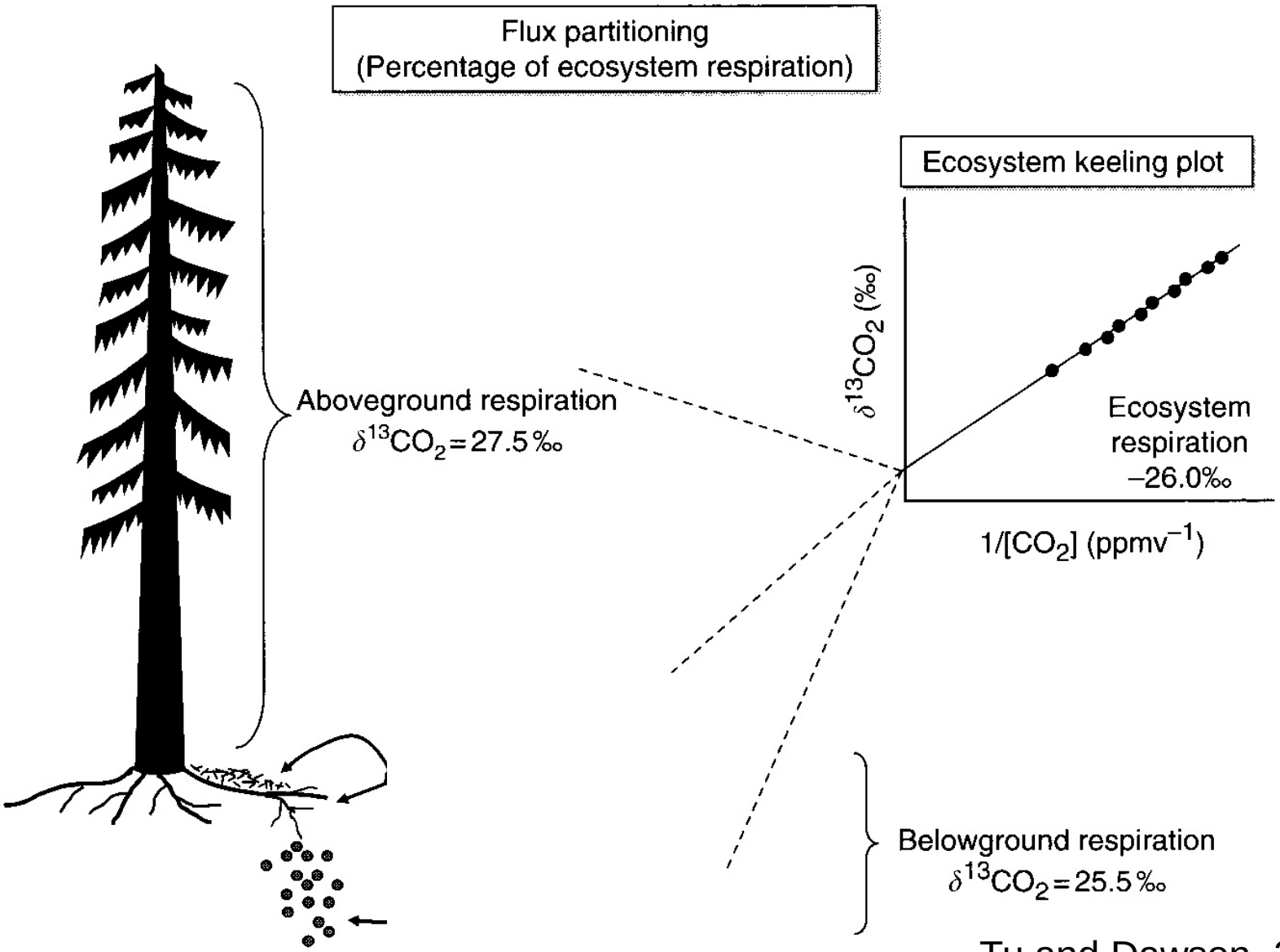
- **Partitioning sources with mixing models**
  - **Two-member mixing model:**

$$\delta^{13}C_{product} = f(\delta^{13}C_{source1}) + (1 - f)*(\delta^{13}C_{source2})$$

$$f_{source1} = (\delta^{13}C_{product} - \delta^{13}C_{source2})/(\delta^{13}C_{source1} - \delta^{13}C_{source2})$$

$$f_{above} = (\delta^{13}C_{ecoresp} - \delta^{13}C_{below})/(\delta^{13}C_{above} - \delta^{13}C_{below})$$

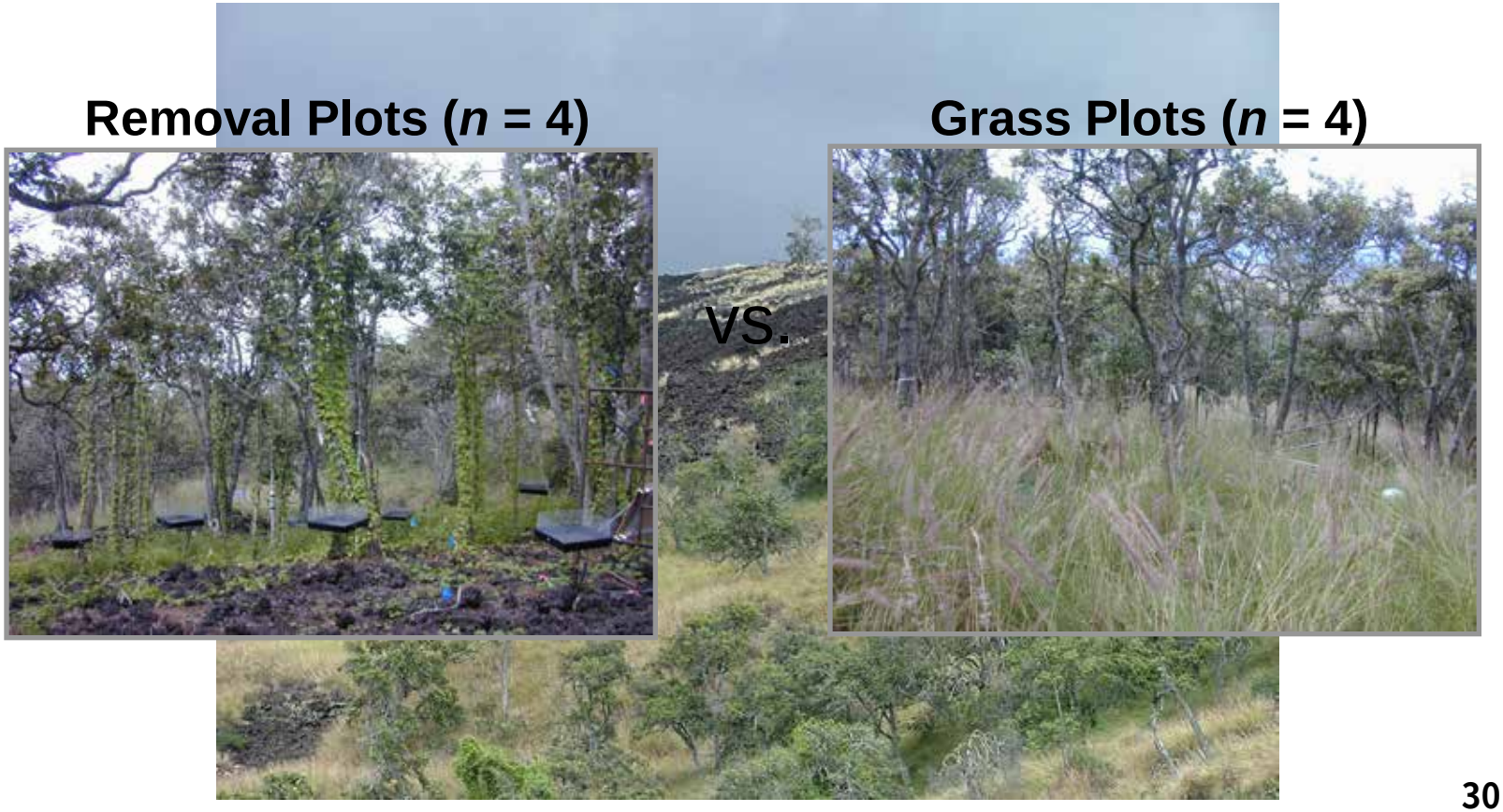
$$f_{above} = (d^{13}C_{ecoresp} - d^{13}C_{below})/(d^{13}C_{above} - d^{13}C_{below})$$



Tu and Dawson, 2005

Utility of C Isotopes

- Impact of C_4 grass invasion into Hawaiian Dry Forest



Utility of C Isotopes

- **Impact of C_4 grass invasion into Hawaiian Dry Forest**
  - **What is the impact of grass invasion on forest soil C cycling?**
  - **C_4 grass invading a C_3 forest**
    - **Used stable C isotopes to partitioning pools and fluxes of soil C into C_4 vs. C_3 components with two-member mixing models (Litton *et al.* 2008)**
      - **Pools: litter, root, and mineral soil C**
      - **Fluxes: Soil CO_2 efflux, and litterfall**

$$f_A = \frac{\bar{\delta}_M - \bar{\delta}_B}{\bar{\delta}_A - \bar{\delta}_B}$$

Utility of C Isotopes

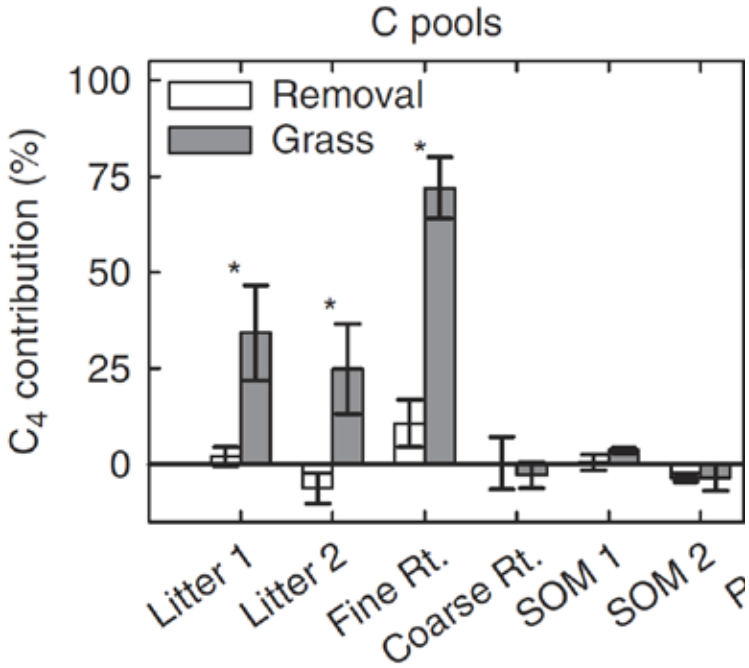
- **Impact of C_4 grass invasion into Hawaiian Dry Forest**
  - **C pool d values (litter, roots, and mineral soil C)**
    - **Sources:**
      - **C_3:**
        - ≫ **Litter C: -23.0 ‰**
        - ≫ **Fine Root C: -22.0 ‰**
        - ≫ **Mineral Soil C: -23.0 ‰**
      - **C_4: -11.0 ‰**

$$f_A = \frac{\bar{\delta}_M - \bar{\delta}_B}{\bar{\delta}_A - \bar{\delta}_B}$$

    - **Products:**
      - **Litter C: -22.5 ‰ (Removal); -18.75 ‰ (Grass)**
      - **Fine Root C: -21.2 ‰ (Removal); -15.4 ‰ (Grass)**
      - **Mineral Soil C: -23.4 ‰ (Removal); -23.4 ‰ (Grass)**

Utility of C Isotopes

- **Impact of C_4 grass invasion into Hawaiian Dry Forest**
  - **Carbon Pools**
    - **C_4 grass contributes a large fraction of litter (25-34%) and fine root C pools (72%), but contributes next to nothing (-3%) to mineral soil C**
      - **How can that be?**
      - **What are the implications for C sequestration?**



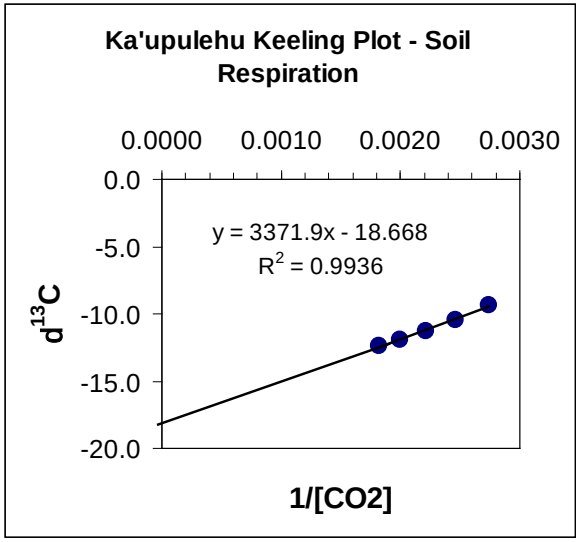
Litton et al., 2008

Utility of C Isotopes

- **Impact of C_4 grass invasion into Hawaiian Dry Forest**
  - **C flux d values & Keeling plot analysis**
    - **Sources:**
      - **C_3: -22.6 ‰ (average of C_3 mineral soil & root C)**
      - **C_4: -12.8 ‰ (average of C_4 mineral soil & root C)**

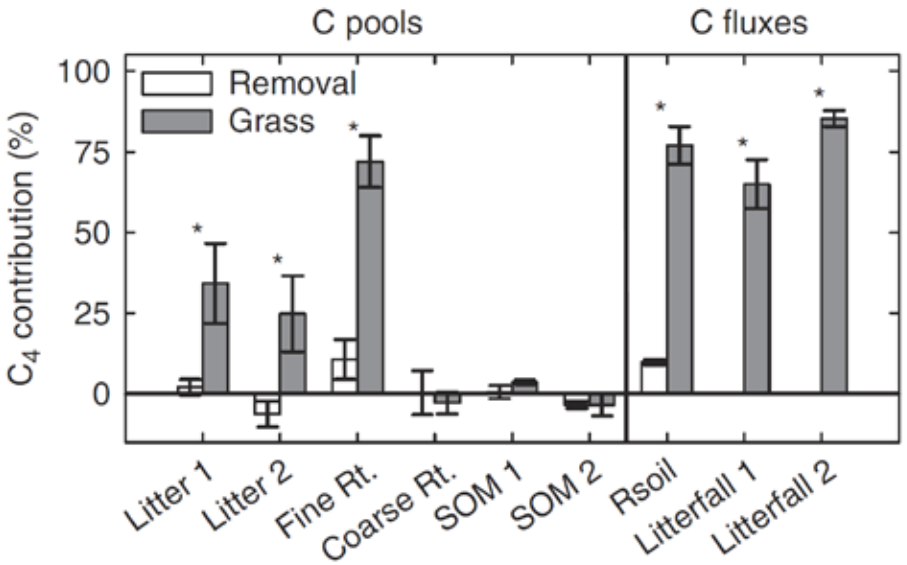
$$f_A = \frac{\bar{\delta}_M - \bar{\delta}_B}{\bar{\delta}_A - \bar{\delta}_B}$$

    - **Product:**
      - **Soil CO_2 Efflux: -18.7 ‰**



Utility of C Isotopes

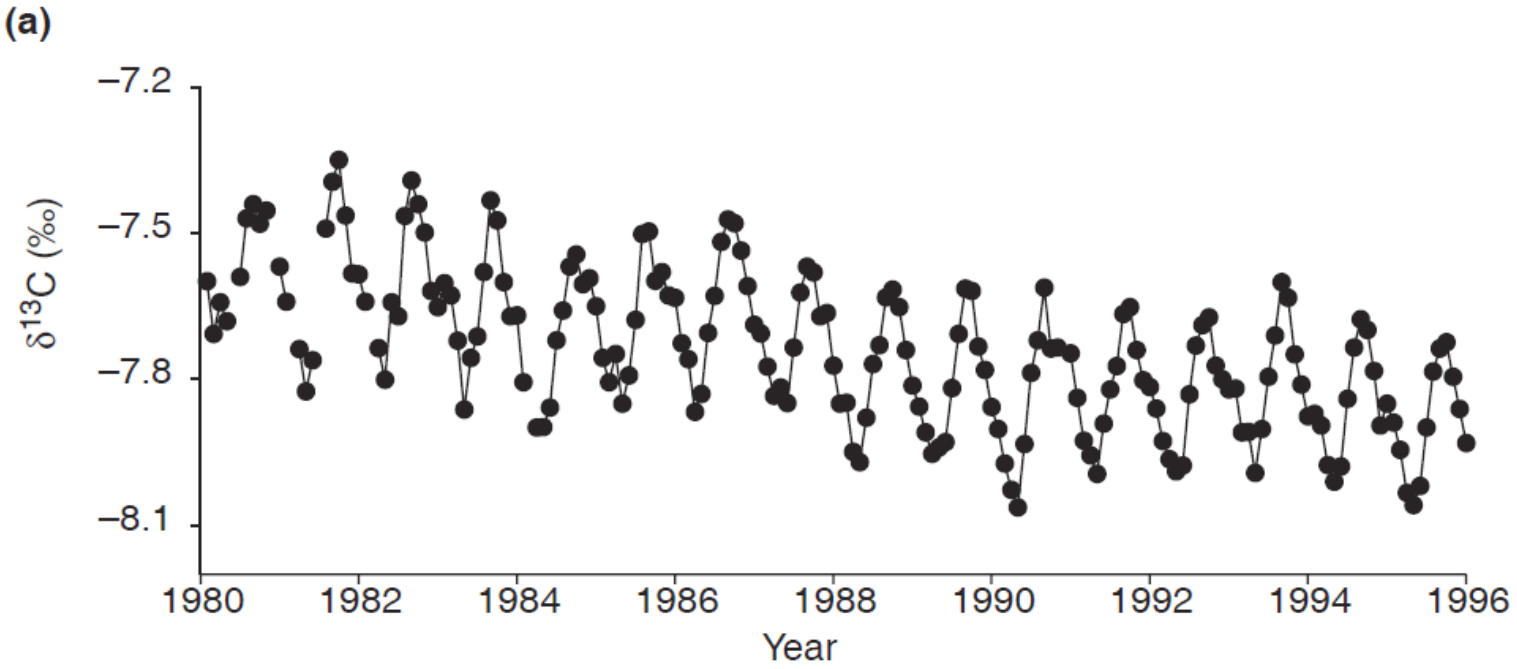
- **Impact of C_4 grass invasion into Hawaiian Dry Forest**
  - **Carbon Flux**
    - **C_4 contribution to soil CO_2 efflux was 10% (Removal) & 77% (Grass)**
    - **C_4 contribution to litterfall in Grass plots was 65-85%**
    - **Grass invasion leads to large increases in flux of C into and out of soils, with no change in soil C storage**



Litton et al., 2008

Utility of C Isotopes

- **Explain this figure, and you understand C isotopes…**



West et al., 2006

Isotope Hydrology

- **Commonly used to trace water sources and fates**
  - **D/^{1}H and ^{18}O/^{16}O**
    - **There are actually 9 different isotopic configurations of H_2O**
      - **H has 2 stable isotopes (H & D), and O has 3 (^{16}O, ^{17}O, & ^{18}O)**
        - **≫^{17}O is very rare, so most H_2O has a mixture of $H_2{}^{16}O$ (common), $H_2{}^{18}O$ (rare), and $HD^{16}O$ (most rare)**
        - **≫Both D and ^{18}O are commonly used in isotope hydrology**

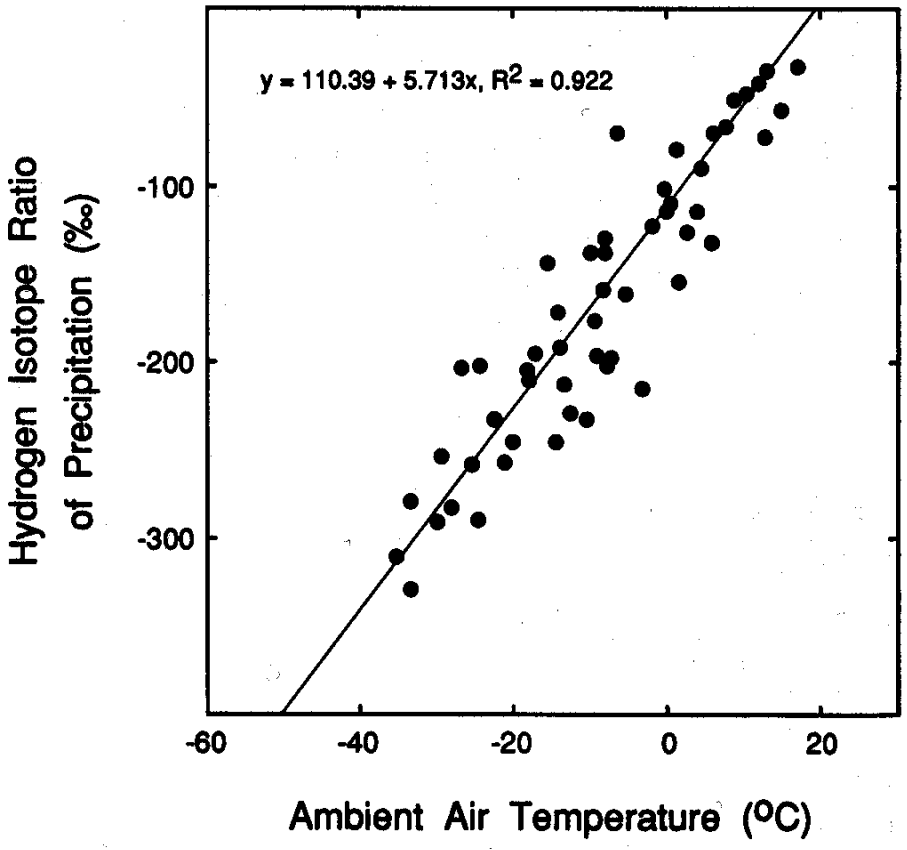
Isotope Hydrology

- **Equilibrium fractionation:**
  - Isotopic exchange reactions between 2 different phases of a compound
  - Example: H_2O vapor $\leftrightarrow$ H_2O precipitation in clouds
  - Although the process is in equilibrium, the rate of exchanges is different so that the result is an enrichment of one of the isotopes in either the source or the product and, therefore, depletion of the isotope in the other

Isotope Hydrology

The Temperature Effect

- **Equilibrium fractionation is temperature dependent**
  - **dD and $d^{18}O$ values in precipitation are strongly related to air temperature during condensation**
  - **Provides a basis for determining seasonality of water use by plants (e.g., with tree rings)**

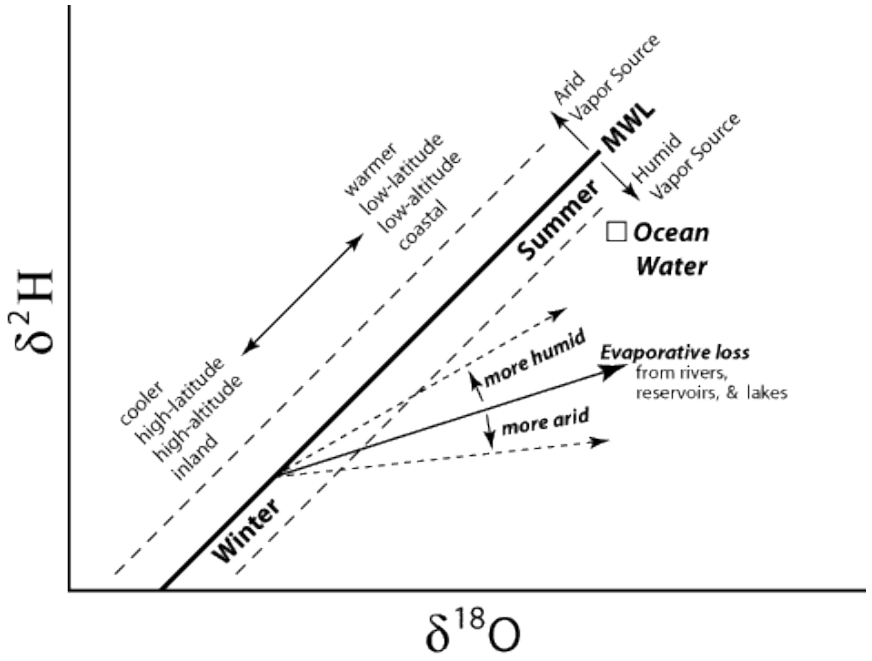


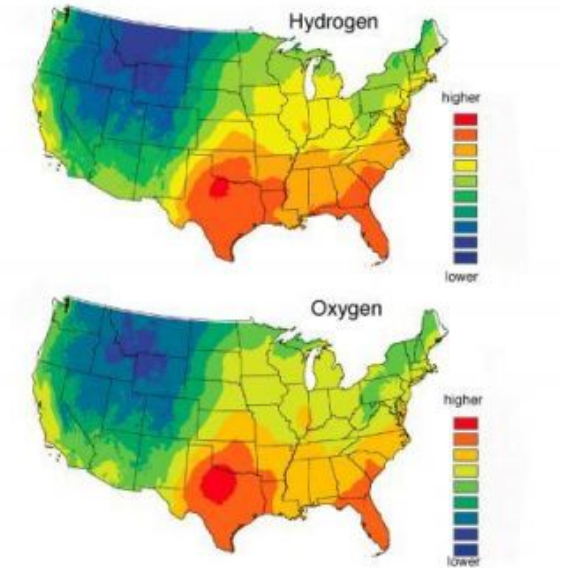
Isotope Hydrology

- **Kinetic fractionation**
  - Unidirectional; equilibrium is not attained
  - Applies to evaporation of surface waters
  - Heavy isotope reacts slower and becomes concentrated in the source
    - And, therefore, depleted in the product

Isotope Hydrology

- Global meteoric water line (GMWL) relates O and H isotopes in freshwater (precipitation)
  - **90% of ocean, ground, and atmospheric water cycles every year**





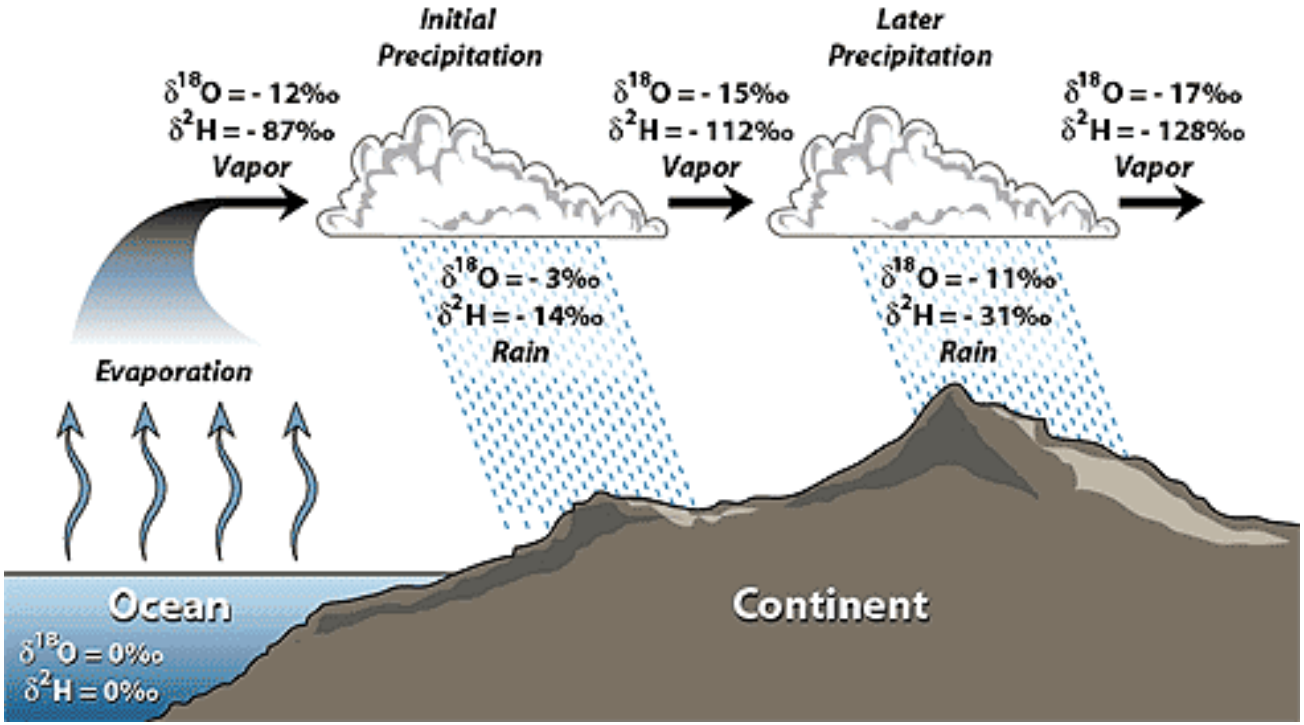
Isotope Hydrology

- You are what you eat – Tracking criminals with D and ^{18}O stable isotopes in hair
  - One hair can be used to determine where you have been drinking water in the last 2 weeks to multiple years
    - **E.g., Origins of unidentified murder victims**
    - **Corroborate / dismiss alibis**

- **Other aspects of criminology**
  - **Sources of drugs (e.g., cocaine and heroin)**
  - **Tracking counterfeit bills to where the cotton was grown**
  - **Does your very expensive bottle of French wine come from France?**
  - **Is your expensive bottle of 100% pure agave tequila really pure agave (C_4; w/ C isotopes)?**

Isotope Hydrology

- Continental and altitudinal effects

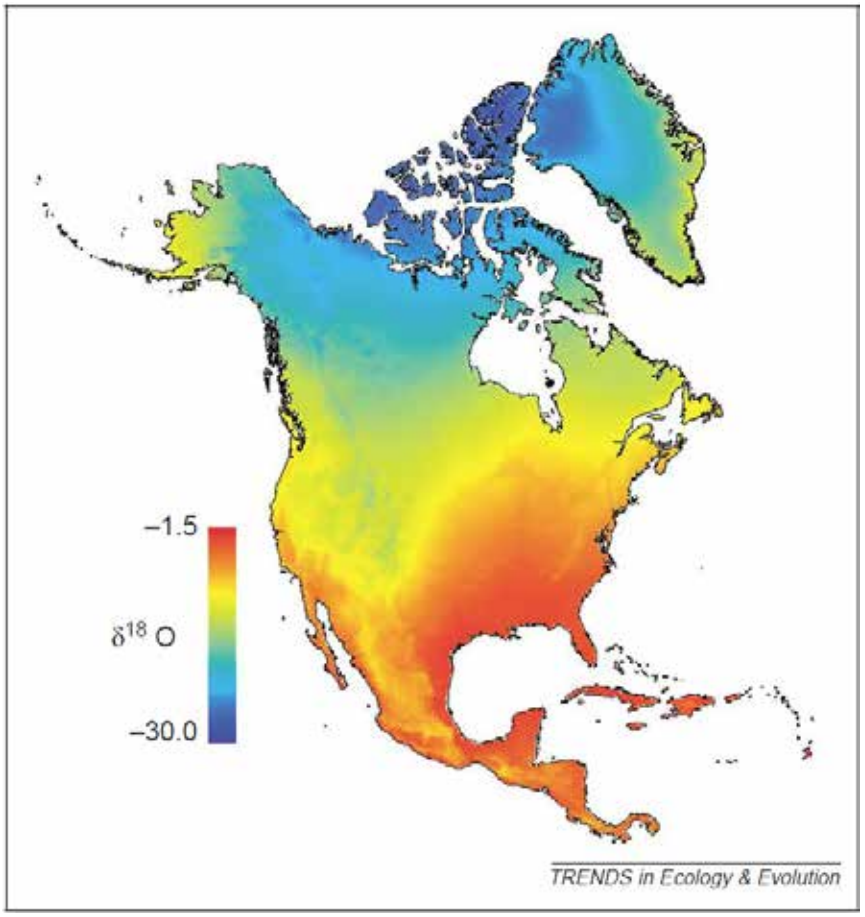


(Hoefs 1997)

- **Amount effect: the heavier the rainfall event, the more depleted the dD and dO values are in precipitation**

Isotope Hydrology

$d^{18}O$ in average annual precipitation



West et al. (2006)

Isotope Hydrology

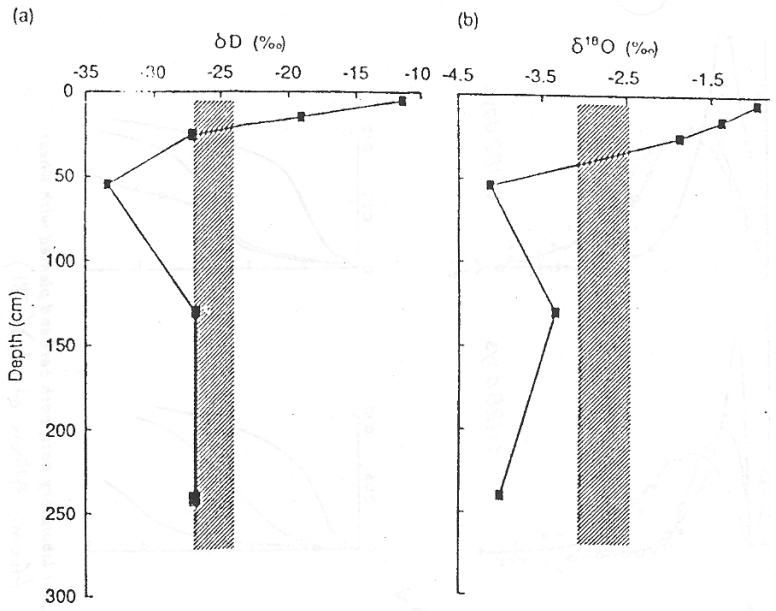
• Differences in source water signatures can be used to examine water use by plants

- **No fractionation of H or O isotopes across the soil-plant continuum (except for halophytes)**

- **Compare isotopic signatures of source water (e.g., soil water, precipitation, cloud water, ground water, etc.) with signature in plant water extracted from the xylem**

Isotope Hydrology

- Differences in source water signatures can be used to examine water use in plants
  - Why do you get gradients in D and ^{18}O isotopes along a soil depth profile?

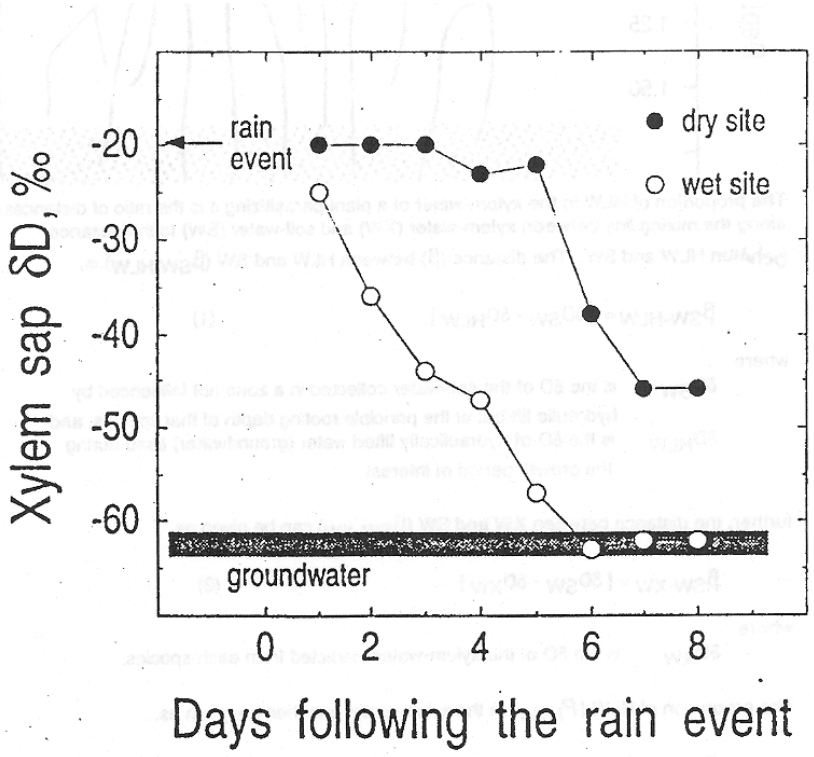


Depth profiles of soil water δD (a) and $\delta^{18}O$ (b) at the inland site in winter (
26), 1991. Hatched area shows the range of tree sap δ values for five sampling times betv
July 24 and September 3.

Thorburn & Walker 1993

Isotope Hydrology

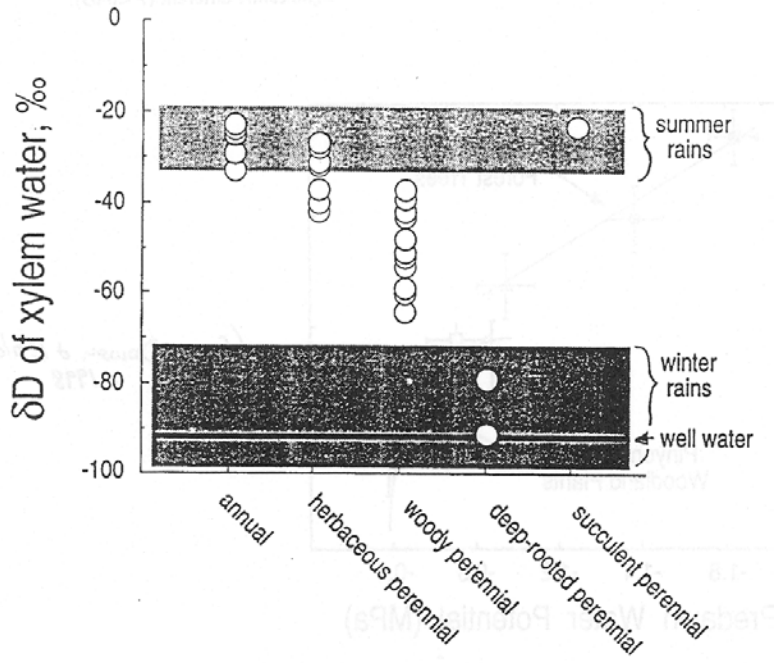
• Differences in source water signatures can be used to examine water use in plants



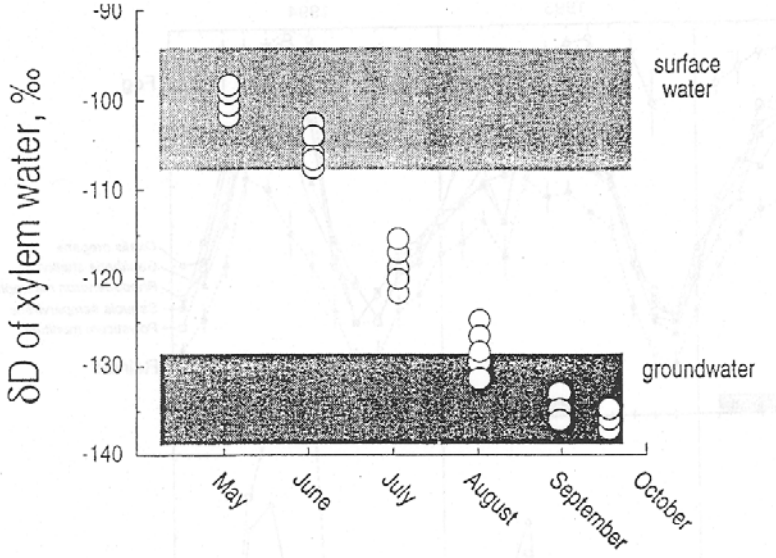
White et al. 1985

Isotope Hydrology

• Differences in source water signatures can be used to examine water use in plants



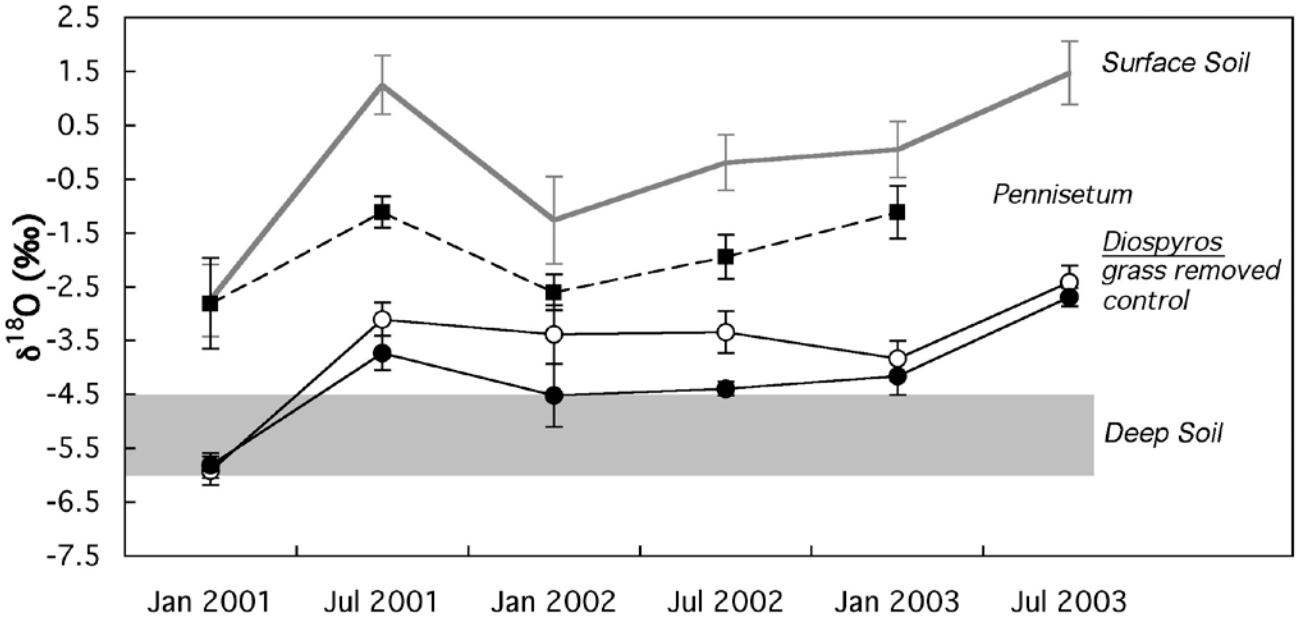
Ehleringer et al. 1991



Smith et al. 1991

Isotope Hydrology

• Differences in source water signatures can be used to examine water use in plants



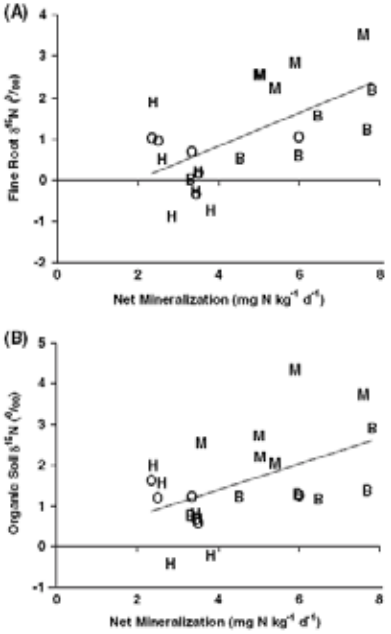
Cordell and Sandquist (2008)

N Isotopes

- ^{15}N has a history of use in pulse-labeling experiments, but is now also being used as a natural tracer

Table 1. *Fractionation factors, α, for various processes in the N cycle*

Process	Fractionation factor
N mineralization (org N → NH_4^+)	≈1·000
$NH_4^+ \leftrightarrow NH_3$ in solution	1·020–1·027*
NH_3 volatilization	1·029
Diffusion of NH_4^+, NH_3, NO_3^- in solution	≈1·000
Nitrification	1·015–1·035
Dentrification	1·000–1·033
N assimilation	1·000–1·020†
N_2-fixation	0·998–1·002
Metabolic steps in plants	0·980–1·020

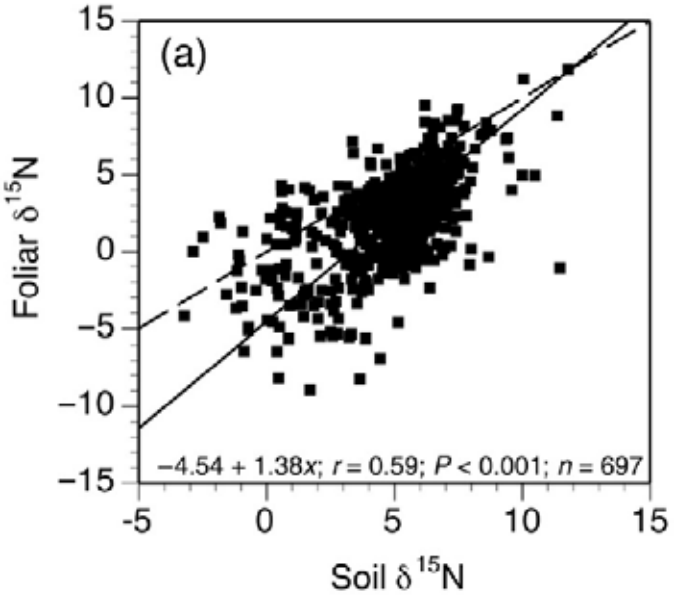


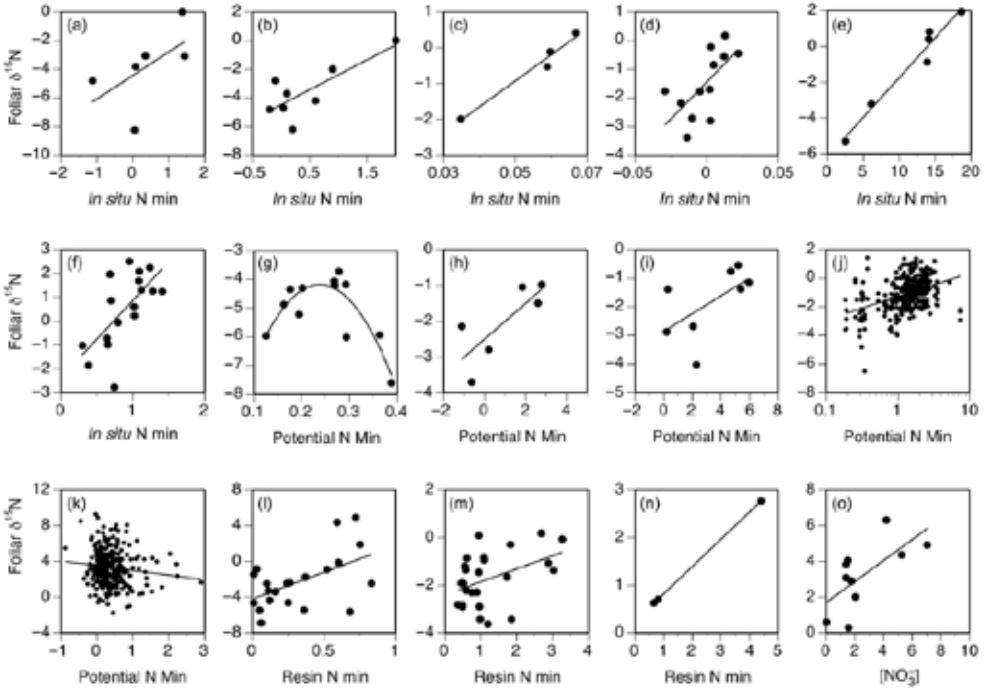
Hogberg 1997

Templer et al. 2007

N Isotopes

- ^{15}N has a history of use in pulse-labeling experiments, but is now being used as a natural tracer

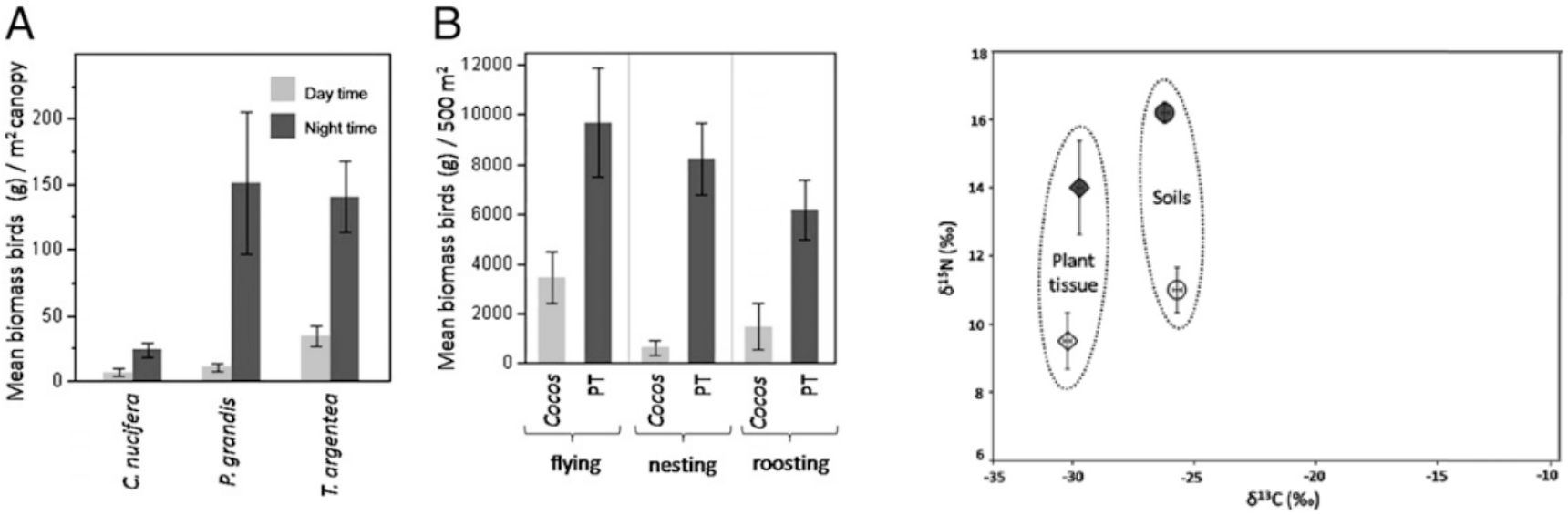




Craine et al. (2009)

N Isotopes

- ^{15}N has a history of use in pulse-labeling experiments, but is now being used as a natural tracer



Young et al. (2010)

N Isotopes

- Alone or combined with C isotopes, N isotopes are now widely used in trophic studies (Tiunov 2007)
  - ^{15}N becomes more enriched (i.e., accumulates) as you move up the food chain
    - Discrimination against ^{15}N in the synthesis & excretion of N metabolites
  - $D\delta^{15}N = \delta^{15}N_{consumer} - \delta^{15}N_{food}$
    - 2.0 - 2.5‰ per trophic level in aquatic & terrestrial ecosystems
  - Trophic fractionation of ^{13}C is insignificant → ^{13}C used to evaluate primary food sources

N Isotopes

- Alone or combined with C isotopes, N isotopes are now widely used in trophic studies (Tiunov 2007)