Stable isotopes in terrestrial ecology – Introduction

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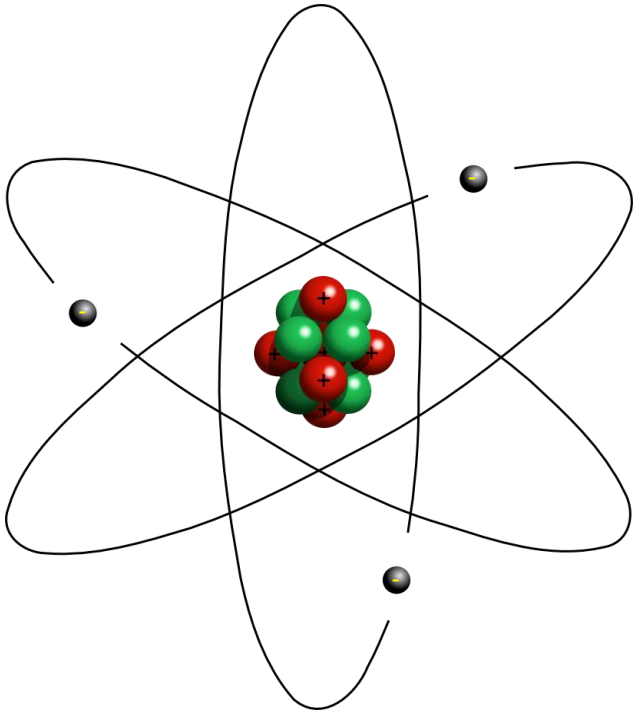
Isotopes

- Atomic structure
  - Every atom consists of protons, neutrons and electrons
  - The number of protons and electrons determine the element (in uncharged atoms the number of protons equals the number of electrons)
  - Atoms of an element with differing numbers of neutrons are called isotopes
    - Nomenclature

$$ {}^{A}_{Z}\mathrm{atom} \quad \text{or} \quad {}^{12}_{6}\mathrm{C},\ {}^{13}_{6}\mathrm{C} $$

      - Z: number of protons
      - N: number of neutrons
      - A: mass number (= Z + N)

    - The number of protons is fixed for every element, therefore the term ^{13}C is sufficient for an unambiguous denomination of an atom
    - $M_Z = 1{,}6726231 \cdot 10^{-27}$ kg
      $M_N = 1{,}6749543 \cdot 10^{-27}$ kg
      $M_e = 9{,}100939 \cdot 10^{-31}$ kg



Isotopes

- Isotopes are atoms of an element with differing numbers number of neutrons
- Isotopes are (almost) undistinguishable in their chemical properties, because these are mostly determined by the electron shell
- However, isotopes differ in some of their physical properties (mass!)
  - E.g.: In a closed volume, the kinetic energy of a gas is given by

$$E_{kin} = \sum m \cdot v^2$$

Light isotopes have the same energy as heavy isotopes

$$\rightarrow \sum m_l \cdot v_l^2 = \sum m_s \cdot v_s^2$$

$$\rightarrow \frac{v_l}{v_s} = \sqrt{\frac{m_s}{m_l}}$$

The differences in the physical properties act out in chemical and biological processes (→ isotope effects)

Isotopes

Hydrogen	Atom weight	^{1}H 1.00782	^{2}H 2.0141	^{3}H $t_{1/2}$ = 13.3 J	
	Relative abundance	99.9885%	0.0115%		
Carbon	Atom weight	^{12}C 12.0000	^{13}C 13.0033	^{14}C $t_{1/2}$ = 5730 J	
	Relative abundance	98.93%	1.107%		
Nitrogen	Atom weight	^{14}N 14.0031	^{15}N 15.0001		
	Relative abundance	99.632%	0.368%		
Oxygen	Atom weight	^{16}O 15.9949	^{17}O 16.9991	^{18}O 17.9991	
	Relative abundance	99.76%	0.038%	0.205%	
Sulfur	Atom weight	^{32}S 31.97207	^{33}S 32.97146	^{34}S 33.96787	^{36}S 35.96708
	Relative abundance	94.39%	0.76%	4.29%	0.02%

Units – atom%

- Abundance (atom%)
  - Frequency of an isotope in 100 atoms of an element

$$\text{atom\%}^{13}\text{C} = \frac{^{13}\text{C}}{^{13}\text{C} + ^{12}\text{C}} \times 100 = \text{F} \times 100$$

	^{12}C	^{13}C	^{14}C
Atom weight	12.0000	13.0033	$t_{1/2}$= 5730 J
Relative abundance	98.93%	1.107%	

$$\text{atom\%}^{18}\text{O} = \frac{^{18}\text{O}}{^{18}\text{O} + ^{17}\text{O} + ^{16}\text{O}} \times 100 = \text{F} \times 100$$

	^{16}O	^{17}O	^{18}O
Atom weight	15.9949	16.9991	17.9991
Relative abundance	99.76%	0.038%	0.205%

  - Is predominantly used in studies with highly enriched tracers

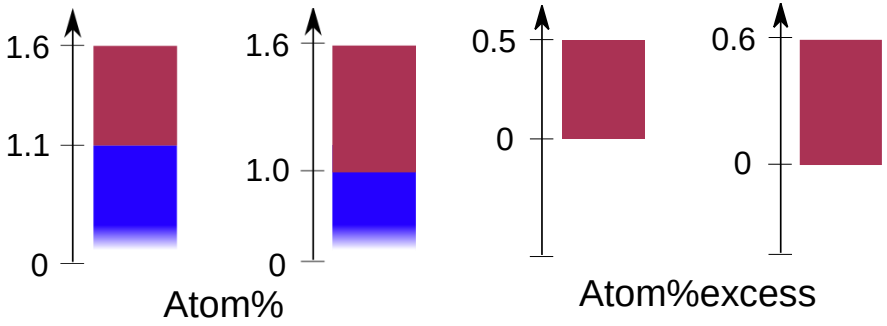
Units - APE

- Atom%excess (APE)
  - Frequency (atom%) above a threshold (base) level

$$\text{atom\%excess} = \left(\left(\frac{^{13}C}{^{13}C + ^{12}C} \right)_{\text{Labelled}} - \left(\frac{^{13}C}{^{13}C + ^{12}C} \right)_{\text{Basis}} \right) \times 100$$

$$= \text{atom\%}_{\text{Labelled}} - \text{atom\%}_{\text{Basis}}$$

  - APE values can easily be used for calculations without the need to subtract „threshold (background) values“
  - Threshold levels can be different for different compartments in one experiment (e.g. resulting from differences in natural abundances due to fractionation)
  - Used in tracer applications
  - APE is a relative value



Units – Delta notation

- Delta-value (δ ‰)
  - Relative value expressed against universally fixed reference values

$$\delta(‰) = \frac{R_{Sample} - R_{Standard}}{R_{Standard}} \times 1000 = \left(\frac{R_{Sample}}{R_{Standard}} - 1 \right) \times 1000$$

with

$$R = \frac{\text{Number of heavy isotopes}}{\text{Number of light isotopes}} = \frac{^{13}C}{^{12}C}$$

$$\left(\text{vgl.} \quad F = \frac{^{13}C}{^{13}C + ^{12}C} = \frac{R}{R+1} \right)$$

R: Proportion

F: Concentration

Units – Delta notation

- Delta-value (δ ‰)
  - Predominantly used to express minimal differences in isotope abundances (especially differences in natural abundance)

    e.g. Carbon 0 ‰ = 1.1057 atom% ^{13}C

    5 ‰ = 1.1111 atom% ^{13}C

  - Isotope abundances are expressed as relative differences between a sample and a reference because the measurement of differences is isotopic frequencies is much easier (and much more accurate) than the determination of an absolute frequency of an isotope in a sample (see measurement techniques)

Units – Delta notation

- Classification of reference substances for the delta values
  - Primary standards
    - (Often) only virtually existent (or exhausted) substances serving as an anchor for the expression of delta values
  - Secondary standards
    - Substances with carefully calibrated or agreed upon values relative to primary standards. Secondary standards are used to calibrate measurement instruments in each laboratory (substances are distributed by IAEA)
  - Working standards
    - Substances calibrated against secondary standards that are used regularly during measurements of unknowns
      e.g. acetanilide or caffeine (for C and N

Units – delta notation

- Reference Substances for expressing delta values (Primary standards)

Carbon: Carbonate (Belemnite) from the PeeDee-formation (V-PDB)

$^{13}C/^{12}C = 0.0111802 \quad \Rightarrow 1.10566$ atom%^{13}C

Calibration is performed *via* the alternative reference substance NBS19 ≡ +1.95‰ vs. V-PDB

Nitrogen: Atmospheric Nitrogen (N_2 Air)

$^{15}N/^{14}N = 0.0036765 \quad \Rightarrow 0.366303$ atom%^{15}N

Oxygen/Hydrogen: Vienna Standard Mean Ocean Water (V-SMOW)

$^{18}O/^{16}O = 0.00200520 \quad \Rightarrow 0.20011872$ atom%^{18}O

(in carbonates. oxygen is often expressed vs. V-PDB)

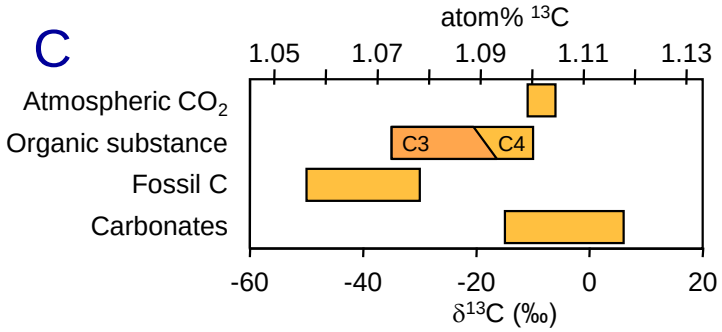
$^{2}H/^{1}H = 0.00015576 \quad \Rightarrow 0.01557357$ atom%^{2}H

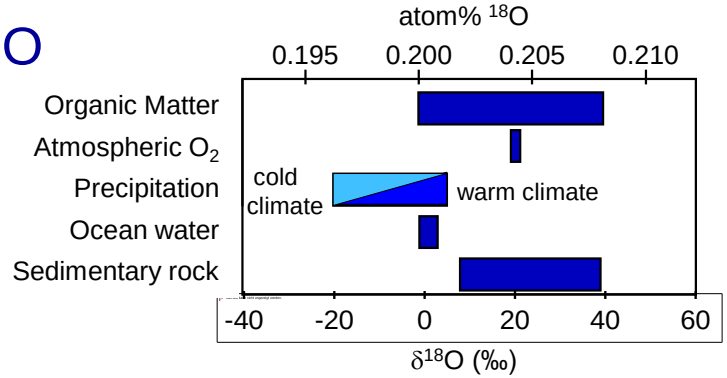
Sulfur: Cañon Diablo Troilit (meteoritic FeS) (CDT)

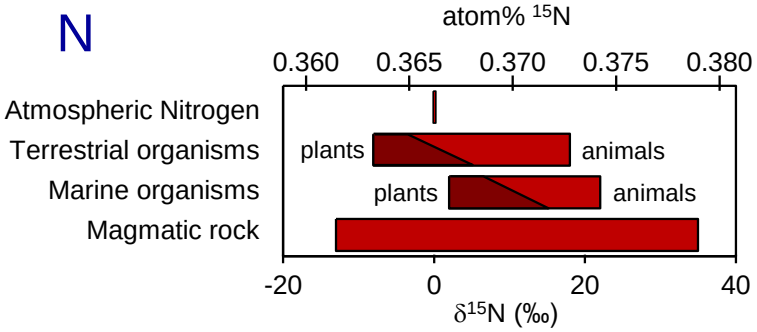
$^{34}S/^{32}S = 0.0450045 \quad \Rightarrow 4.306632$ atom%^{34}S

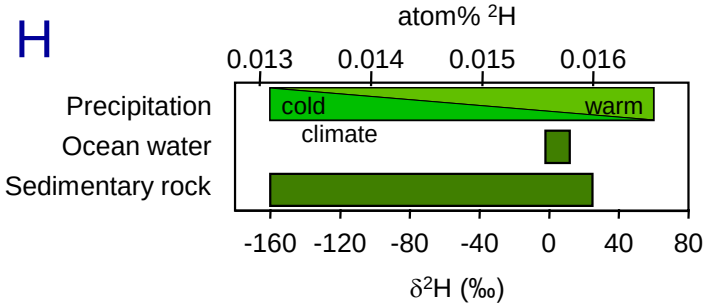
Calibration is performed *via* the alternative reference substance IAEA-S-1 ≡ -0.30‰ vs. CDT

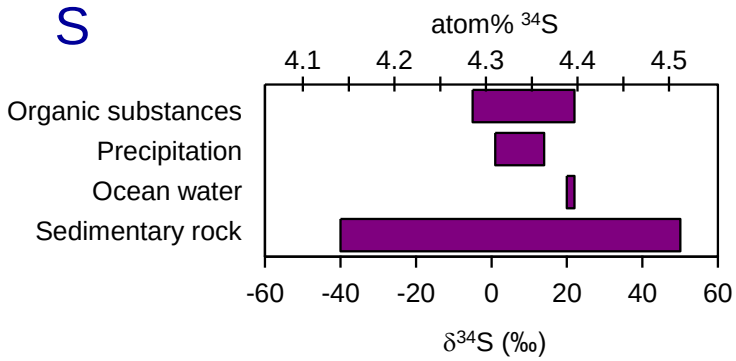
Ranges of natural abundance











- Comparision of 0.01 atom% difference for different elements
  - ^{13}C 0.01 atom% ≅ 9.15 ‰
  - ^{15}N 0.01 atom% ≅ 27.4 ‰
  - ^{34}S 0.01 atom% ≅ 2.43 ‰
  - ^{18}O 0.01 atom% ≅ 50.03 ‰
  - ^{2}H 0.01 atom% ≅ 642.3 ‰

Units in isotope abundance – Delta-value

- Conversion of delta values between reference scales

Ususally unknowns (sa = sample) are measured against a laboratory or working standard (wstd = working standard). This working standard is calibrated against a primary standard (pr. Std. = primary standard).
In order to receive values that can be compared among different laboratories, $\delta_{sa/wstd}$ values must be converted to $\delta_{sa/pr.Std}$ values.

$$\delta_{sa/pr.Std.} = \left(\frac{R_{sa}}{R_{pr.Std.}} - 1\right) * 1000\ ‰$$

$$\delta_{sa/pr.Std.} = \left[\left(\frac{\delta_{sa/wstd}}{1000} + 1\right) * \left(\frac{\delta_{wstd/pr.Std.}}{1000} + 1\right) - 1\right] * 1000\ ‰$$

$$\delta_{sa/pr.Std.} = (\delta_{sa/wstd} + 1000) * \left(\frac{\delta_{wstd/pr.Std.}}{1000} + 1\right) - 1000\ ‰$$

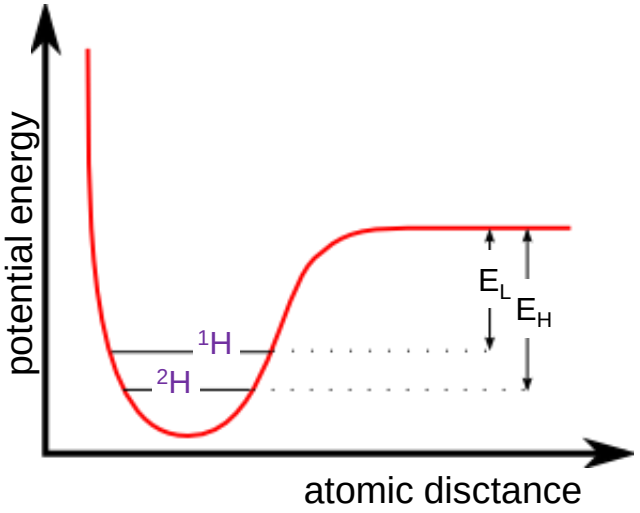
$$\delta_{sa/wstd} = \left(\frac{R_{sa}}{R_{wstd}} - 1\right) * 1000\ ‰ \quad \Rightarrow R_{sa} = \left(\frac{\delta_{sa/wstd}}{1000} + 1\right) * R_{wstd}$$

$$\delta_{wstd/pr.Std.} = \left(\frac{R_{wstd}}{R_{pr.Std.}} - 1\right) * 1000\ ‰ \quad \Rightarrow R_{pr.Std.} = \frac{1}{\left(\frac{\delta_{wstd/pr.Std.}}{1000} + 1\right)} * R_{wstd}$$

$$\delta_{sa/pr.Std.} = \delta_{sa/wstd} + \delta_{wstd/pr.Std.} + \frac{\delta_{sa/wstd} * \delta_{wstd/pr.Std.}}{1000}\ ‰$$

Fractionation

- Isotopes of an element differ in some physical properties, e.g.
  - atomic mass
  - zero point energy, which is deteremined by the vibrational motion of the atoms
    - Chemical bonds with heavy isotopes are stronger (Dissociation energy E_H is greater than E_L)
    - Light isotopes are mor „agile“ (as a result of vibrational motion)

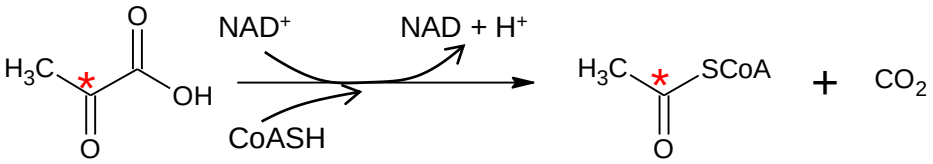


- Differences in physical properties of isotopes result in differences in chemical and biological processes
  - The ratio between light and heavy isotopes differs in different pools (e.g. between substrate and product of a chemical reaction)

    → Fractionation, isotope effect

Fractionation

- Kinetic isotope effect
  - Light and heavy isotope differ in **reaction rates**



$\left(\frac{^{12}k}{^{13}k}\right)_{C^*}$ = 1.0232 k: reaction rates

  - Occurs with fast, incomplete or unidirectional reactions
  - Is usually bigger than steady state isotope effect

Fractionation

- Thermodynamic (steady state-) isotope effect
  - Occurs with steady state reactions when the **reaction equilibrium** is different for light and heavy isotopes

$$CO_2(g) + H_2O \rightleftharpoons H_2CO_3$$

$$\left(\frac{^{12}K}{^{13}K}\right)_{0°C} = 0.9897 \qquad \left(\frac{^{12}K}{^{13}K}\right)_{30°C} = 0.9930$$

$$K = \frac{c(H_2CO_3)}{c(CO_2) * c(H_2O)}$$

Steady state constant

  - Is usually smaller than kinetic isotope effects
  - Bond strength has a strong impact:
    Heavy isotopes accumulate where bonds are strongest.

    → heavy isotopes are usually depleted in reaction products
  - Is temperature dependent: Usually decreases with increasing temperature

Fractionation

- Basic rules
  - Isotopic fractionation decreases with increasing temperature
    - Differences in activation energy play a lesser role at higher temperatures (=higher energy content)
  - Isotopic fractionation is highest for light elements
    - Relative mass difference is higher for light elements

- Processes resulting in fractionation
  - Evaporation, Condensation
  - Melting, solidifying (because isotopes differ in melting point, surface tension, viscosity, melting heat, heat of formation)
  - Diffusion (different gradient of concentration and mass)
  - Gravitational forces (enrichment of U^{235} in centrifuges)
  - Photochemical reactions (e.g. assimilation of CO_2; different excitation/ionisation wave numbers)
  - Different equilibrium states in chemical reactions (different reaction heat, bond strength)

Fractionation

- Fractionation is the base requirement for all natural abundance studies
- Fractionation may impede tracer experiments
- Examples for fractionation for natural processes

e.g.

$\delta_P - \delta_S = -54‰$

H_2O (l) $\rightleftharpoons$ H_2O (g)

$\delta^2H = -15‰$ $\delta^2H = -69‰$

H	$\delta_{product}-\delta_{substrate}$
transition H_2O liquid – gaseous (50°C)	-54‰
transition H_2O solid - liquid	-21.2‰
C	
CO_2 fixation by RuBisCO (photosynthesis)	-29.0‰
CO_2 diffusion	-4.4‰
transitiion CO_2 (g) – H_2CO_3 (30°C)	-7‰
N	
N_2 fixation (Leguminoses)	-3 to +1‰
NH_4^+ assimilation (field)	-10‰
NO_3^- assimilation (field)	-5‰
NH_3 gaseous - NH_4^+ aq	-25‰
O	
transition H_2O liquid – gaseous (50°C)	-8‰
transition H_2O - HCO_3^- aq (25°C)	30‰
transition H_2O - CO_2 gaseous	42.5‰
S	
reduction sulfate -sulfide	0 to -46‰

more at:
http://www.ggl.ulaval.ca/cgi-bin/isotope/generisotope.cgi

Fractionation

- The fractionation factor α indicates the magnitude of the isotope effect

For the reaction $CO_2 + H_2O \rightleftharpoons H_2CO_3$

it is defined as $\alpha_{(^{13}C)} = \frac{R_{CO_2}}{R_{H_2CO_3}}$ with the isotope ratio $R = \frac{\text{number of heavy isotopes}}{\text{number of light isotopes}}$

For irreversible reactions $\alpha = \frac{k_l}{k_s}$, for steady state reactions $\alpha = \frac{K_l}{K_s}$

where k_l and k_h are the reaction rates, K_l and K_s the rate constants for light and heavy istopes, respectively

(α is bigger than 1, when the light isotope is reacting faster or prefers the product side over the substrate)

Fractionation

- The fractionation factor α indicates the magnitude of the isotope effect

For the reaction $CO_2 + H_2O \rightleftharpoons H_2CO_3$

it is defined as $\alpha_{(^{13}C)} = \frac{R_{CO_2}}{R_{H_2CO_3}}$ with the isotope ratio $R = \frac{\text{number of heavy isotopes}}{\text{number of light isotopes}}$

- Example:

$CO_{2\,(air)} + H_2O \rightleftharpoons H_2CO_3$

substrate product

$\delta^{13}C$ = -8.00 ‰ $\delta^{13}C$ = -0.97‰ $\delta_{product}$-$\delta_{substrate}$ = 7.03‰

R = 0.011091 R = 0.011169 $\alpha = \frac{0.011091}{0.011169} = 0.9930$

Fractionation

- Another expression for fractionation is the **Isotope enrichment factor** $\varepsilon = (\alpha - 1) \cdot 1000$
- The advantage of using ε is that the difference between two pools is given in delta permil (like the delta values) and is thus easier to handle
- For small ε the following approximation is valid: $\delta_P = \delta_S - \varepsilon \quad \Leftrightarrow \quad \varepsilon = \delta_S - \delta_P$

  the correct expression is: $$\varepsilon = \frac{\delta_s - \delta_P}{1 + \frac{\delta_P}{1000}}$$

- Also a common expression is the „discrimination“ (which refers to the degree to which a reaction “avoids” the heavy istope)

  $\Delta = \delta_P - \delta_S$

- The symbols ε and Δ are not always used in a consistent manner in the literature

Fractionation

- Examples
  - **CO_2-diffusion:**

$CO_{2\ (air)}$ substrate ⇔ $CO_{2\ (stoma)}$ product

$\Delta_{diffusion} = \delta_P - \delta_S = -4.40$ ‰

$\delta^{13}C_{(air)} = -8.00$‰ ➔ $\delta^{13}C_{(stoma)} = -12.40$‰

$$\varepsilon = \frac{\delta_s - \delta_P}{1 + \frac{\delta_P}{1000}}$$

$\varepsilon = 4.455$

$\varepsilon = (\alpha - 1) \cdot 1000$
$\alpha = \varepsilon/1000 + 1$

$\alpha = 1.004455$

  - **CO_2-fixation by RubisCO (photosynthesis):**

$CO_{2\ (air)}$ substrate ⇔ $C_6H_{12}O_{6\ (plant)}$ product

$\Delta_{RubisCO} = \delta_P - \delta_S = -29.00$ ‰

$\delta^{13}C_{(air)} = -8.00$‰
➔ $\delta^{13}C_{(plant)} = -37.00$‰

$\varepsilon = 30.11423$

$\alpha = 1.03011423$

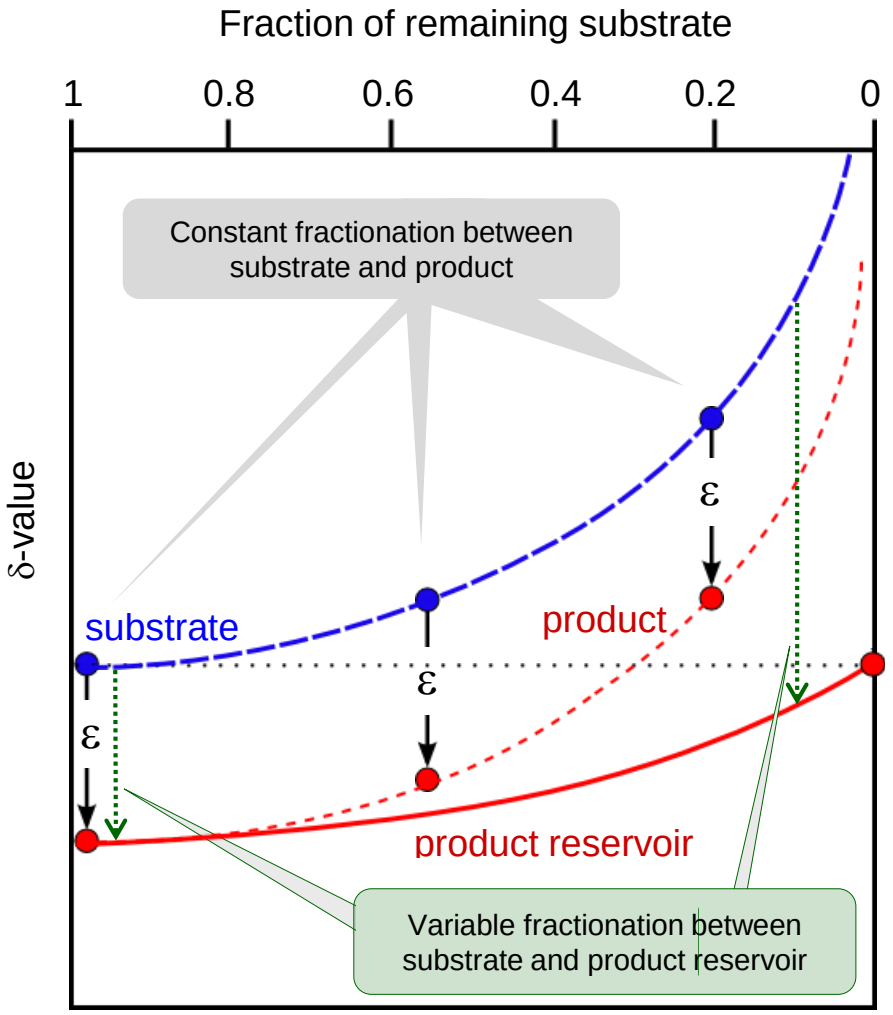
Fractionation in closed systems

- Isotope fractionation can only manifest, if a reaction (which is subject to fractionation) remains incomplete.

closed system: S → P

- Discrimination of the heavy isotope leaves the substrate enriched in heavy isotopes
- Product formed later in the reaction process becomes increasingly heavy
- As a result, the isotopic composition of the product reservoir converges to that of the initial substrate
- Once the reaction has completed (and one single product has been formed), the resulting product has the same isotopic composition as the initial substrate (since it is composed of the very same isotopes)

→ Rayleigh-distillation



Fractionation

- The isotopic composition of the product reservoir can be calculated as follows (with *f* as fraction of remaining substrate)

$$R_{Substrate} = R_{0-Substrate}\, f^{(\alpha-1)}$$

in delta notation

$$\ln\left(\frac{\delta_{Substrate}+1000}{\delta_{0-Substrate}+1000}\right) = (\alpha-1)\ln f = \frac{\varepsilon}{1000}\ln f$$

for small ε

$$\delta_{Substrate} - \delta_{0-Substrate} \cong \varepsilon \ln f$$

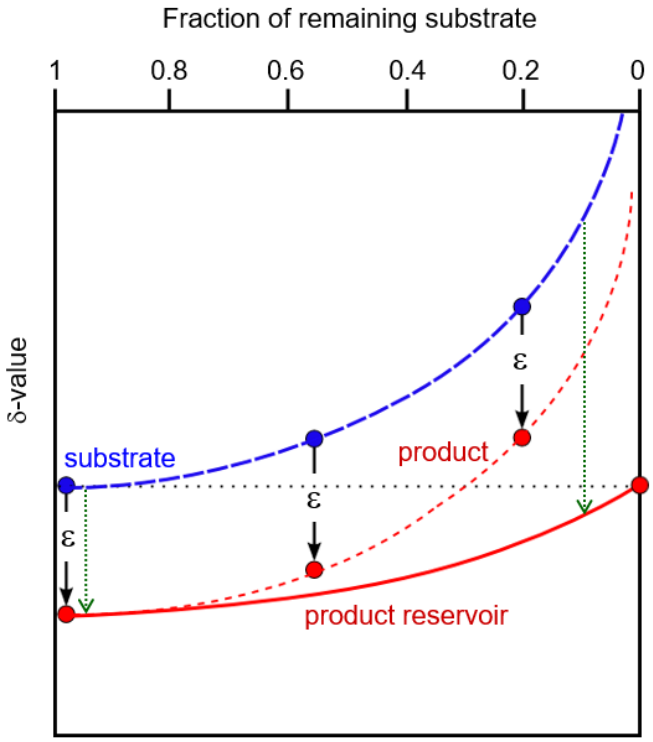
with the mass balance

$$\delta_{0-Substrate} = f \cdot \delta_{Substrate} + (1-f)\cdot \delta_{Product\ reservoir}$$

follows

$$\delta_{Product\ reservoir} = \delta_{Substrate} - \frac{\varepsilon \ln f}{1-f}$$

$$\delta_{Product\ reservoir} = \delta_{0-Substrate} - \frac{f \cdot \varepsilon \ln f}{1-f}$$

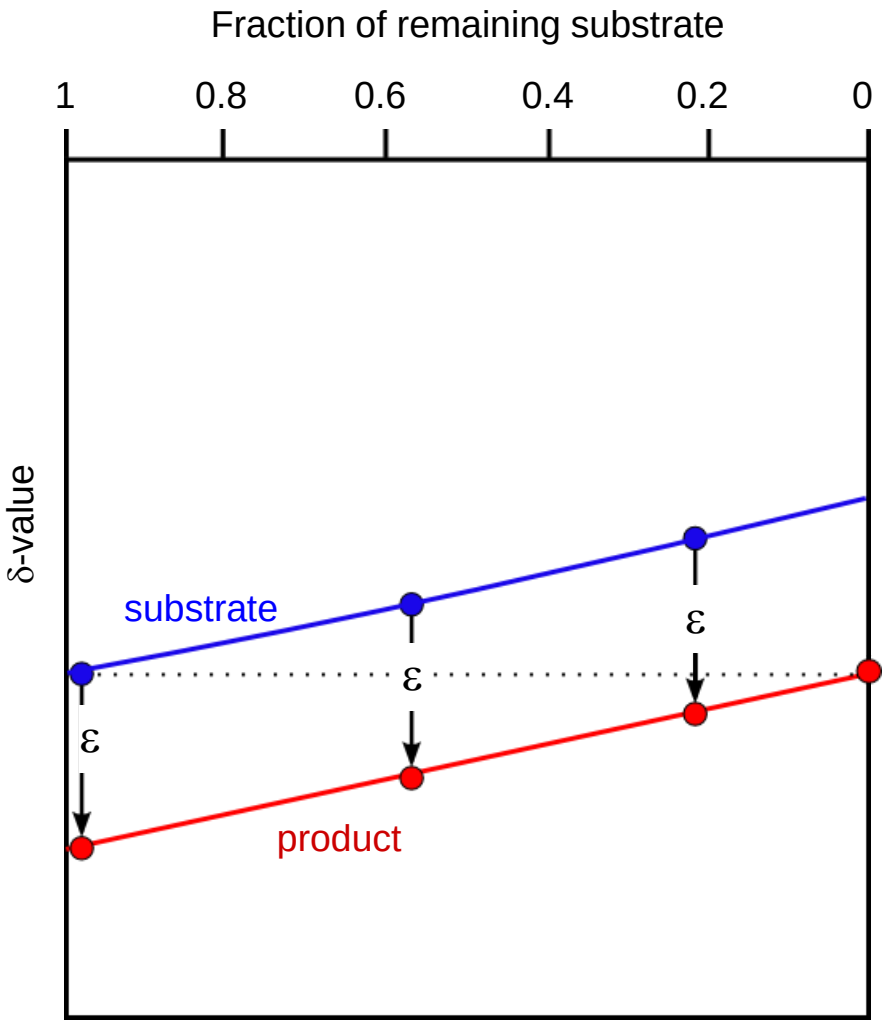


Fractionaction in open systems

- Isotope fractionation can only manifest, if a reaction (which is subject to fractionation) remains incomplete.

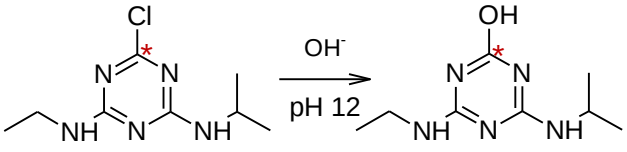
open system: S → Q
S → P

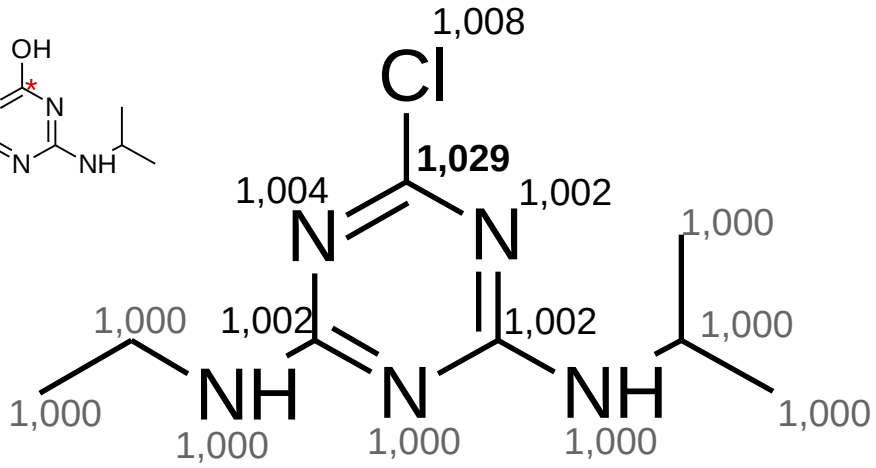
  - In a open system, fractionation depends on the fraction of product formation (the remaining fraction Q leaves the system unchanged)
  - A higher fraction of product formed
    - increases changes in isotopic composition of the substrate
    - decreases differences between product and initial substrate
  - If all substrate is converted to one single substrate, there is no fractionation (but also no „open“ system)



Fractionation during chemical reactions

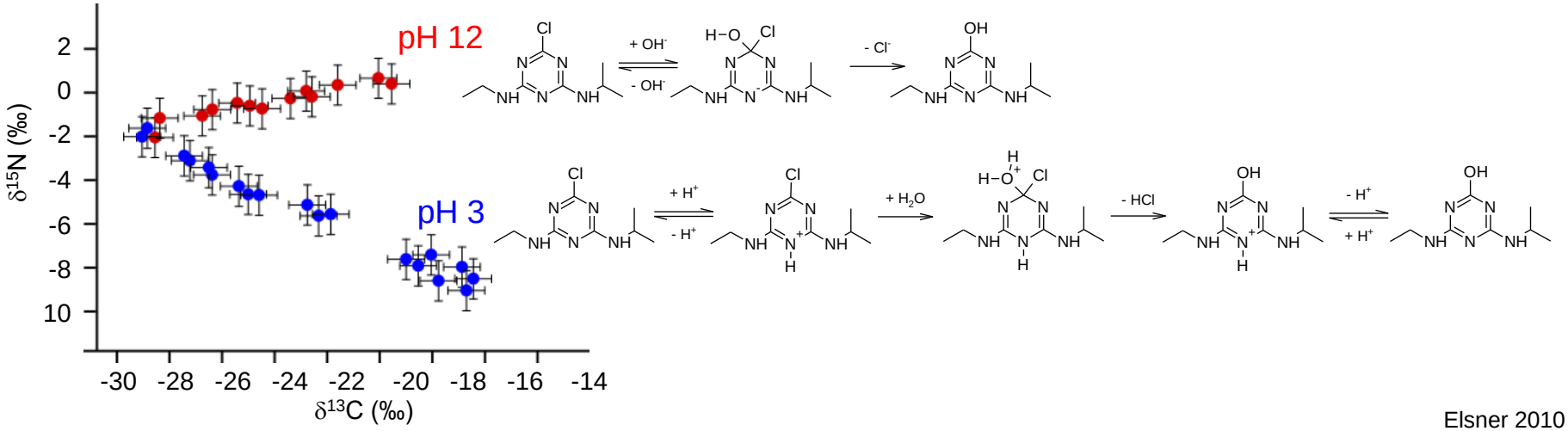
- Fractionation during a chemical reaction is created at the reactive center(s) of the reaction
- The primary isotope effect is bigger than the secundary isotopeo effect
- The apparent fractionation decreases with increasing molecule size since fractionation at the reactive center is „diluted“ by uninvolved atoms (of the same element)





Fractionation during chemical reactions

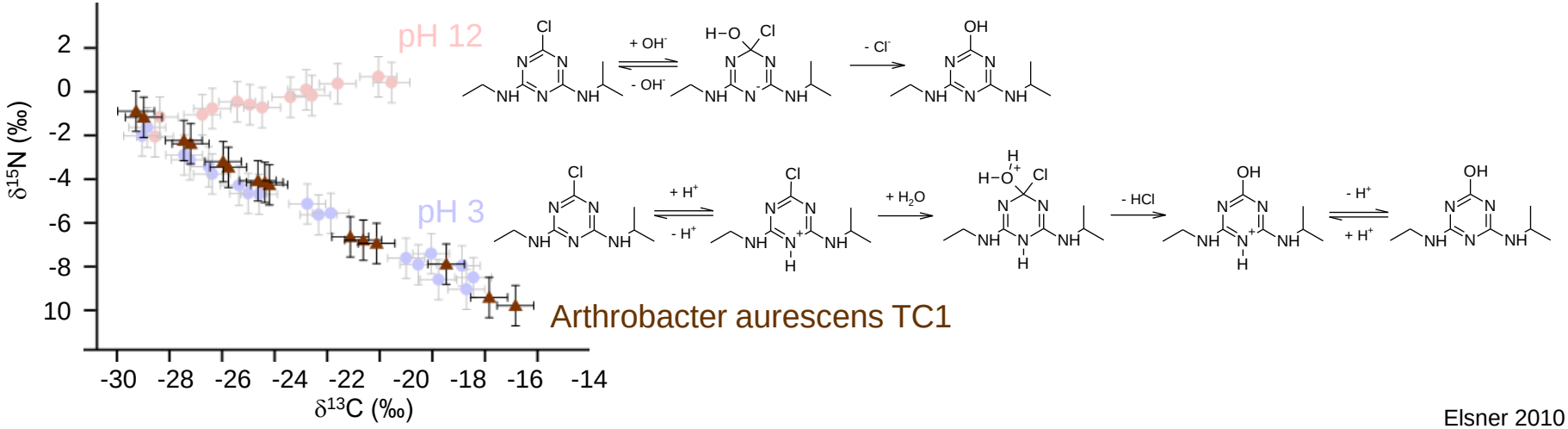
- From the fractionation during reactions the reaction mechanism can be inferred
  - at pH 12
    - Isotope effect for N is considerably smaller than for C: N is affected by secondary isotope effects only,
      as N is not directly involved in the reaction
    - Isotope effect is positive for C and N (light isotopes react faster, as their bonds are broken more easily)
  - at pH 3
    - Isotope effect for C and N have similar size: Both C and N are directly involved in the reaction
    - Isotope effect positive for C, but negative for N at pH 3 (Protonation of N leads to stronger binding in N → transition state is more stable → higher chance of product formation)



Elsner 2010

Fractionation during chemcial reactions

- Comparison of fractionation during enymatic reactions in microorganisms and abiotic reactions (with known reaction mechanism) shows, which mechanism is employed during enzymatic reaction
- Reaction mechanisms are discernible even if the same product is formed by different mechanisms
- The mechanism of degradative reactions is discernible even if the product is not recoverable because the isotope effect is (also) observable in the reactant (educt)
  - In this case, the degradative product can possibly be inferred from the isotope effect analysed in the reactant even if the product cannot be analysed (e.g. because it quickly reacts to other products or is volatile)



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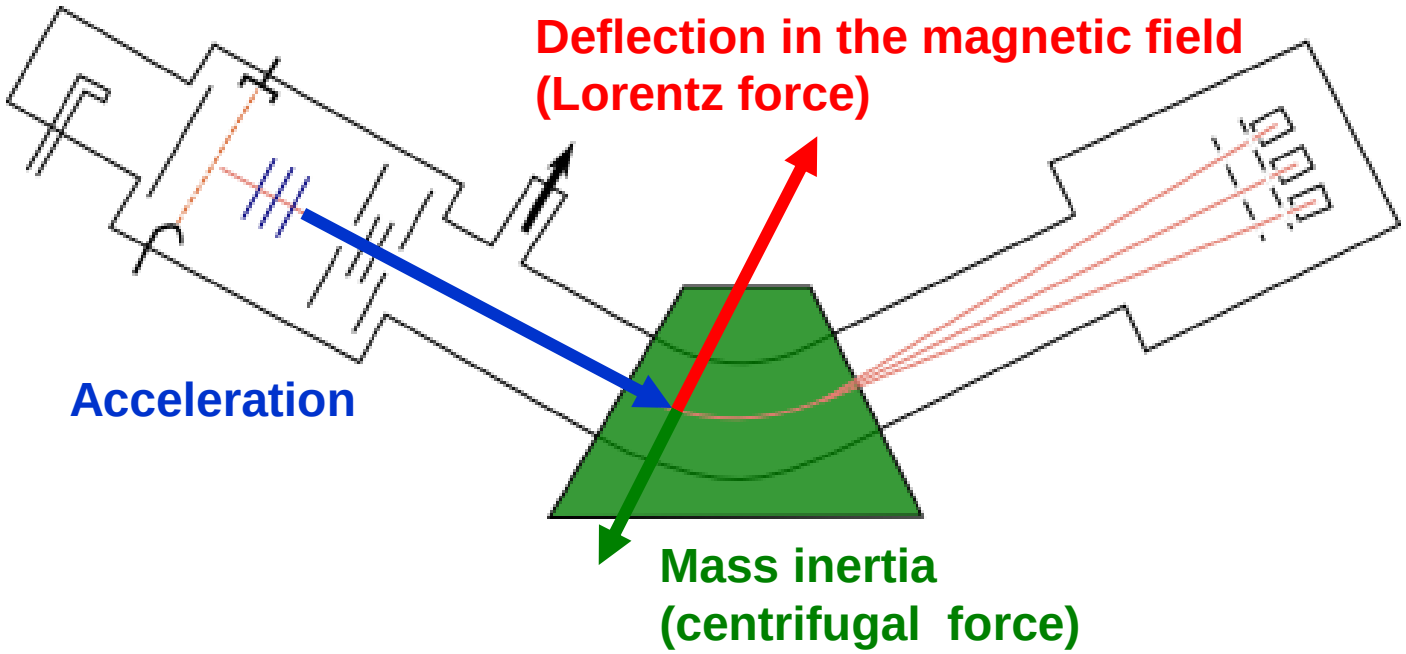
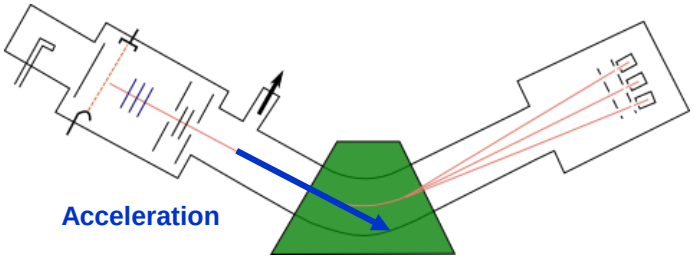
Measurement techniques – theory

- Basis of mass spectrometry
  - Accelerated charged particles are deflected in a magnetic field. The deflection radius is higher for particles of higher masses.



Theory



Forces in the electric field (Acceleration)

The electrical energy $\mathbf{A_{el}}$ of an ion after passing a potential difference U equals

$$A_{el} = q\,U$$

with A_{el} = Energy [J]
q = Electric load [As, C]
U = Voltage [V]

The kinetic energy $\mathbf{A_{kin}}$ of a mass m equals

$$A_{kin} = \tfrac{1}{2}\,m\,v^2$$

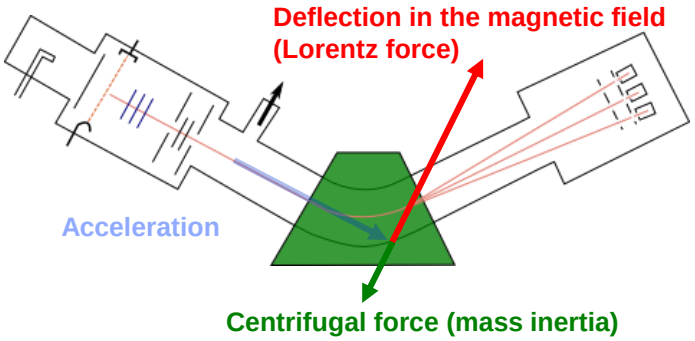
with m = Mass [kg]
v = Velocity [m/s]

If the electric energy is completely converted into kinetic energy, it yields

$$A_{el} = A_{kin}$$
$$q\,U = \tfrac{1}{2}\,m\,v^2$$

$$\Rightarrow v = \sqrt{2\frac{q \cdot U}{m}}$$

Theory



Forces in the magnetic field (deflection)

$$\vec{F}_{magn} = q \cdot \vec{v} \cdot \vec{B}$$

Lorentz force

with $\vec{F}_{magn}$ = magnetic force

$\vec{B}$ = magnetic induction

$\left(\vec{B} = \mu\mu_0\vec{H}, \quad \vec{H} = \text{magnetisation force}\right)$

The vector of F is perpendicular to v und B (right hand rule).

Thus the Lorentz force induces no acceleration but a change of direction.

The centrifugal force acts in the opposite direction

$$\vec{F}_{zentr} = m\frac{v^2}{r}$$

centrifugal force (mass inertia)

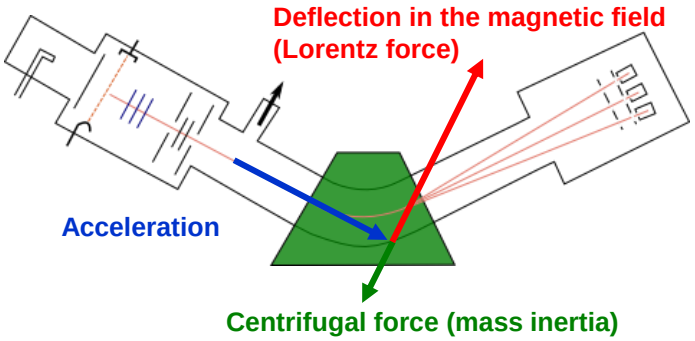
with m = mass [kg]

v = velocity [m/s]

r = radius of the orbit [m]

$$\vec{F}_{centr} = \vec{F}_{magn} \quad \Rightarrow q \cdot v \cdot B = m\frac{v^2}{r}, \qquad \Rightarrow \mathbf{r} = \frac{\mathbf{m} \cdot \mathbf{v}}{\mathbf{q} \cdot \mathbf{B}}$$

Theory



Combination of electric and magnetic fields

from $v = \sqrt{2\frac{q \cdot U}{m}}$ (acceleration in the electric field)

and $r = \frac{m \cdot v}{q \cdot B}$ (deflection in the magnetic field/mass inertia)

follows

$$r = \left(\frac{m}{q \cdot B}\right)\sqrt{2\frac{q \cdot U}{m}} \Rightarrow r = \sqrt{\frac{m}{q}}\sqrt{2U}\frac{1}{B}$$

$$\Rightarrow \frac{r\,B}{\sqrt{2U}} = \sqrt{\frac{m}{q}}$$

For $m_2 > m_1$ follows $r_2 > r_1$ (with $q_1 = q_2$)

→ heavy particles are deflected less

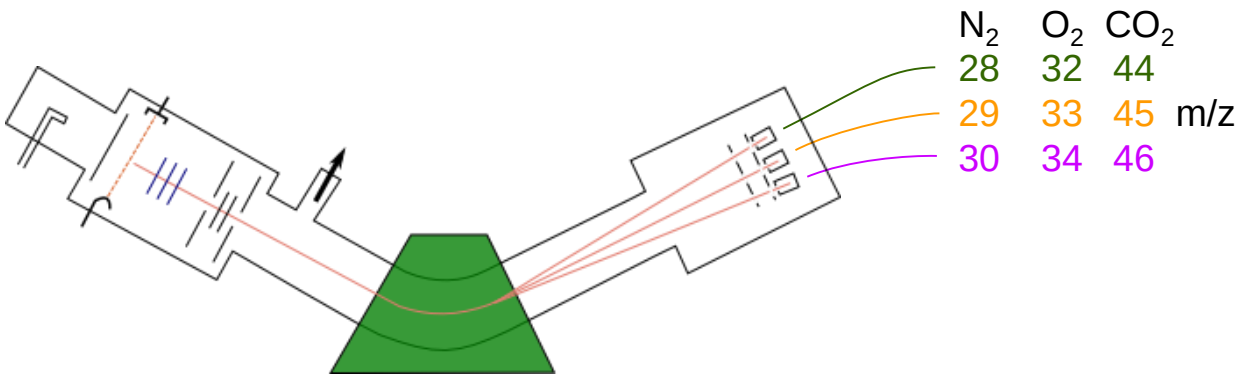
Mass spectrometer

- IRMS (Isotope Ratio Mass Spectrometry)
  - Heavy and light isotopes of one sample are detected in parallel in different detector cups
    ≠ „regular“ mass spectrometer where different masses are detected sequetially
  - The parallel detection of all masses cancels fluctuations in ion sequestration, acceleration voltage, magnetic field etc.

→ much higher accuracy as with sequential mass determination

  - Results are expressed relative to a working standard which has been calibrated against a secondary standard

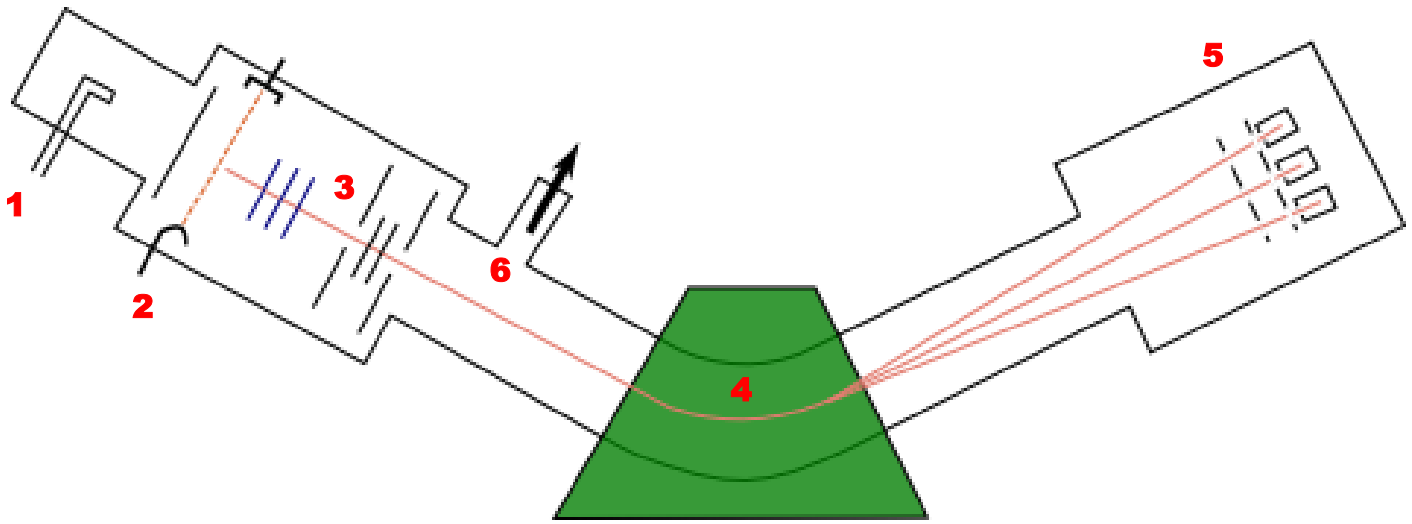
→ only relative differences between samples are determined

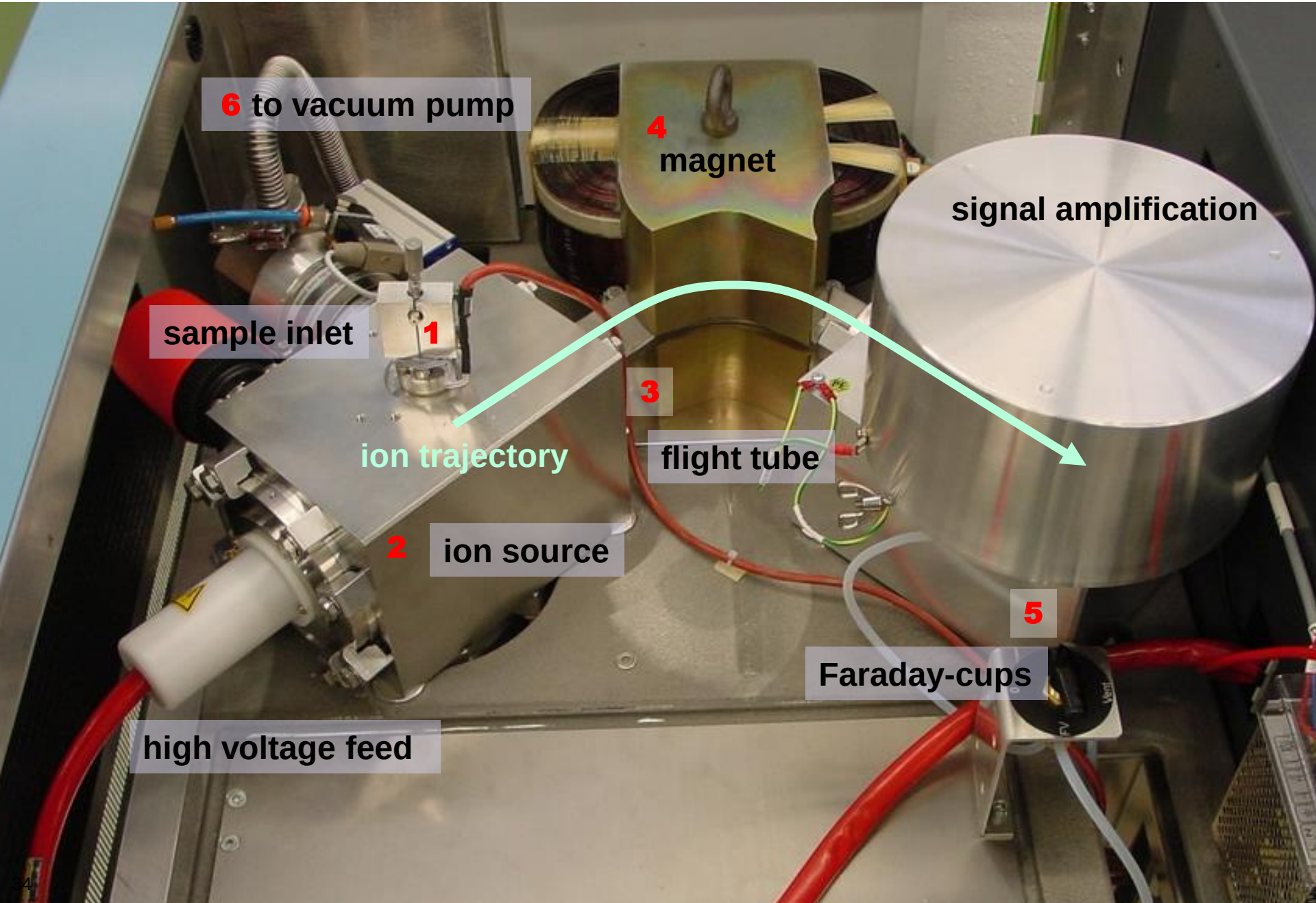


Mass spectrometer

- Scheme of a mass spectrometer

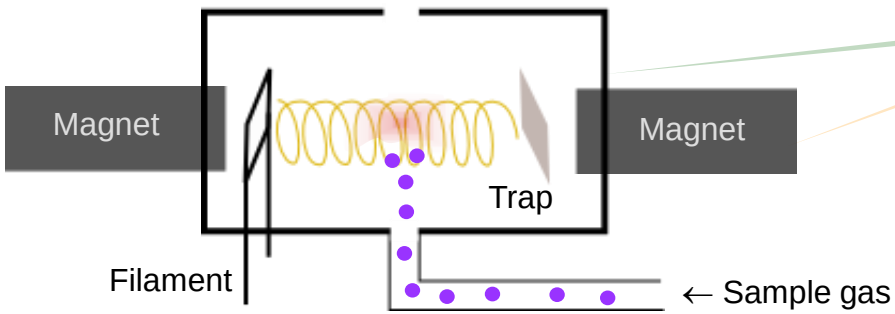
1. Sample inlet
2. Ion source (ionisation of sample molecules)
3. Acceleration and focussing of ions in the flight tube
4. Deflection in the magnetic field
5. Detection of ions separately for each mass in in Faraday-Cups
6. The complete flight tube is under high vacuum (10^{-7} to 10^{-9} mbar), to minimise particle collisions

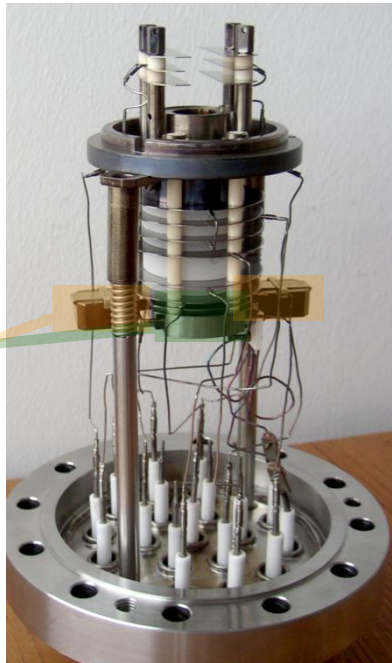



6 to vacuum pump
4
magnet
signal amplification
sample inlet
1
3
ion trajectory
flight tube
2
ion source
5
Faraday-cups
high voltage feed

Mass spectrometer

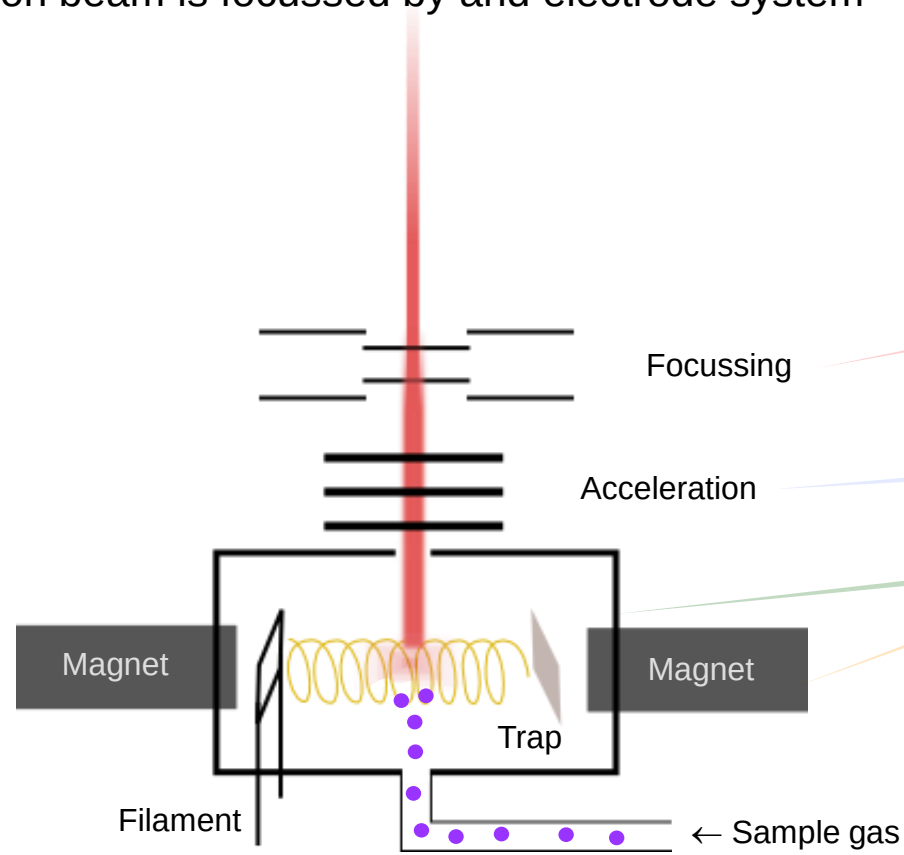
- Ion generation
  - Electrons are expelled from a heated tungsten filament and accelerated towards the trap plate (approx. 100V between filament and trap)
  - Electrons are forced into a circular path by applying a magnetic field. This is to increase the probability of collision with a sample molecule (to ~1‰)
  - Sample gas ions are formed by the collision with an electron (e.g. N_2^+, CO_2^+, …)

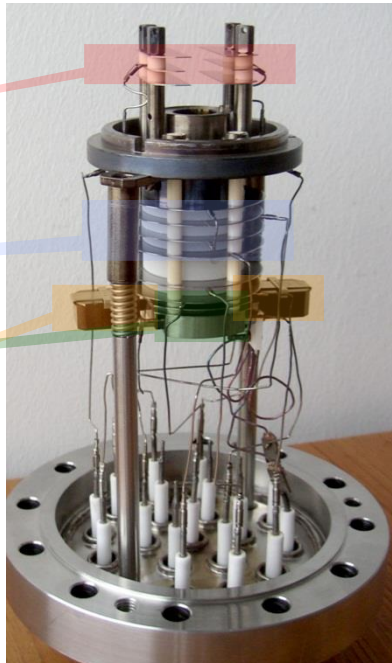




Mass spectrometer

- Ion focussing
  - Ionised molecules are accelerated by application of high voltage (3-10 kV) into the mass spectrometer
    (with 3kV, a CO_2^+-ion will be accelerated to $1.15 \cdot 10^7$ cm/s = 414 000 km/h)
  - The ion beam is focussed by and electrode system

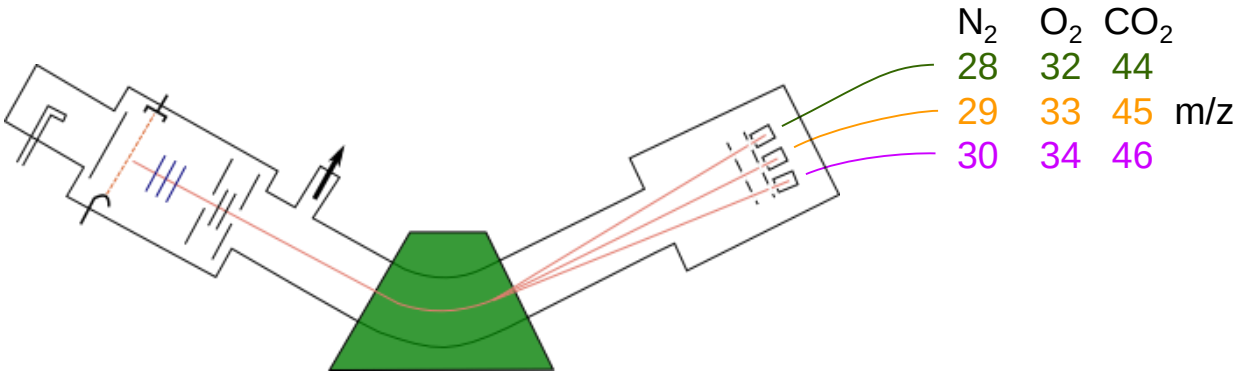




Mass spectrometer

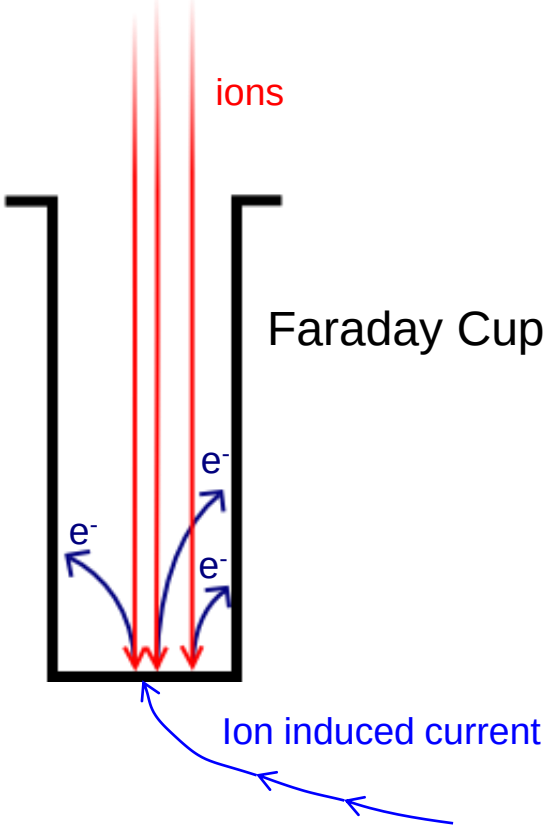
- Ion deflection in the magnetic field
  - Electromagnets are tuneable to different masses (e.g. m/z = 28,29,30; 44,45,46)
  - Ions of different masses (i.e. different isotopic composition) are deflected onto different orbits and detected separately.
  - The detection of different masses in parallel cancels fluctuations in ionisation, acceleration, deflection etc.

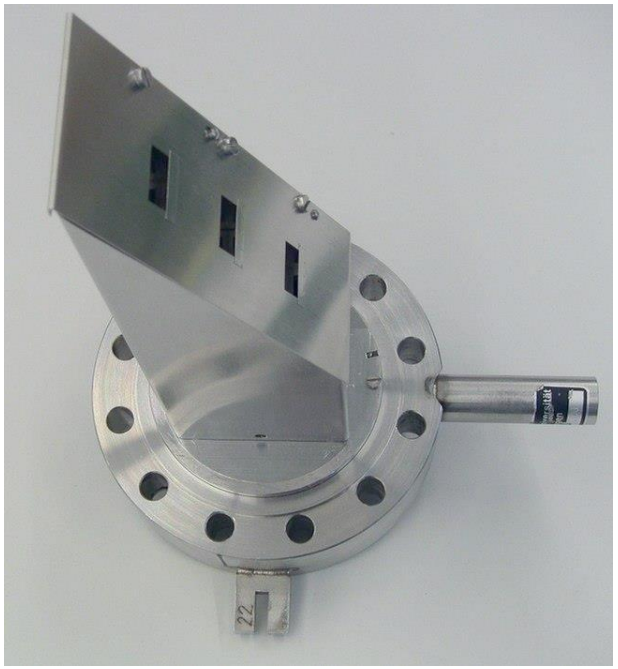
→ much higher accuracy as with sequential mass determination



Mass spectrometer

- Detection of ions in Faraday-Cups
  - Ions are trapped in Faraday Cups. The surface acts as a dynode, i.e. it emits an electron for every trapped ion. The emitted electrons are amplified and detected as a current.
    The rare (heavy) masses are amplified more than the abundant masses.





Mass spectrometer

- Isotope abundance is calculated form the number of molecules trapped in the Faraday Cups
  - For the determination of N:

    m = 28: $^{14}N_2$

    m = 29: $^{14}N^{15}N$

    m = 30: $^{15}N_2$ (and $^{14}N^{16}O$ as contamination from the combustion or from sample gas fragmentation in the ion source)

    - Since the abundance of $^{15}N_2$ is extremely small in natural samples (0.0013%), the contamination by NO has a relatively high impact and thus the abundance of $^{15}N_2$ cannot be determined precisely
    - For most analyses, abundance of $^{15}N_2$ can be calculated from the abundance of $^{14}N^{15}N$
    - However, e.g. denitrification processes with tracer application entail a non-equilibrium between $^{14}N^{15}N$ und $^{15}N_2$
      - For equilibration, microwaves are used to destroy all N_2 molecules. During subsequent reformation of N_2, ^{14}N and ^{15}N are distributed stochastically among the molecules and are thus in equilibrium (**Microwave equilibration**).

Mass spectrometer

- Isotope abundance is calculated form the number of molecules trapped in the Faraday Cups
  - For the determination of C or O:
    - CO_2 has a number of isotopologues (í.e. molecules of equal mass but different isotopic composition)

m = 44: $^{12}C^{16}O_2$

m = 45: $^{12}C^{16}O^{17}O$, $^{13}C^{16}O_2$

m = 46: $^{12}C^{16}O^{18}O$, $^{12}C^{17}O^{17}O$

m = 47: $^{12}C^{18}O^{17}O$, $^{13}C^{16}O^{18}O$, $^{13}C^{17}O_2$

m = 48: $^{12}C^{18}O_2$, $^{13}C^{17}O^{18}O$

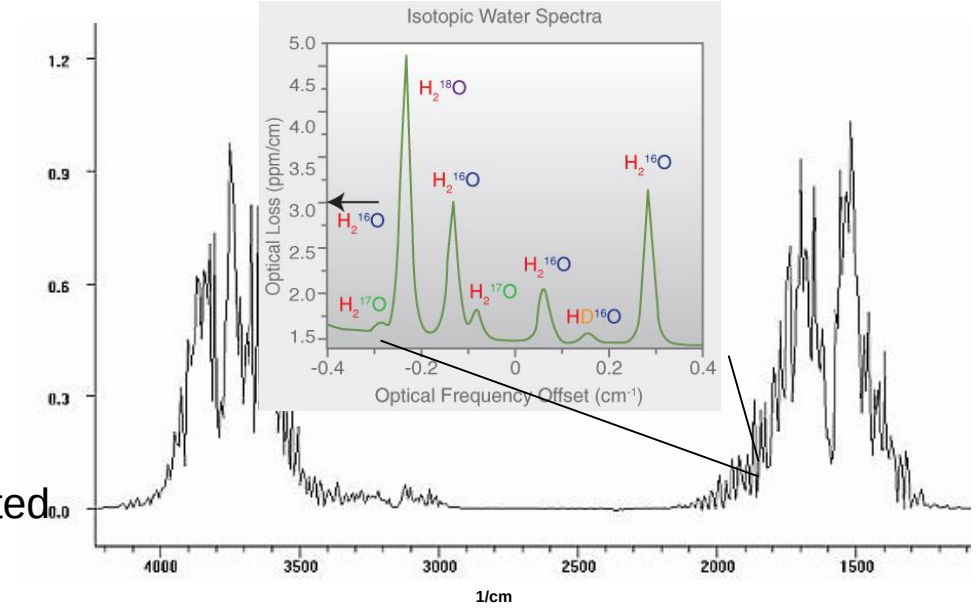
m = 49: $^{13}C^{18}O_2$

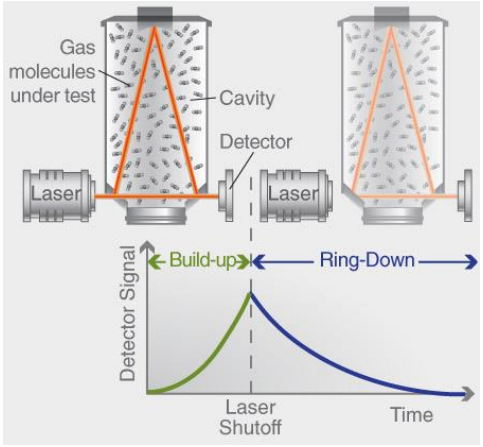
- Isotopologues with more than one rare isotope (^{13}C, ^{17}O, ^{18}O) can be neglected
- To calculate the abundance of $^{13}C^{16}O_2$, the signal of mass 45 is corrected by the abundance of $^{12}C^{16}O^{17}O$ (which is calculated from the abundance of $^{12}C^{16}O^{18}O$)

Alternatives to mass spectrometry

- IR-Spectroscopy (Cavity Ring Down Spectroscopy) for CO_2, N_2O or H_2O
  - Small molecules with a variable or inducible dipol moment (e.g.CO_2, H_2O, but not N_2) absorb infrared radiance to oscillate

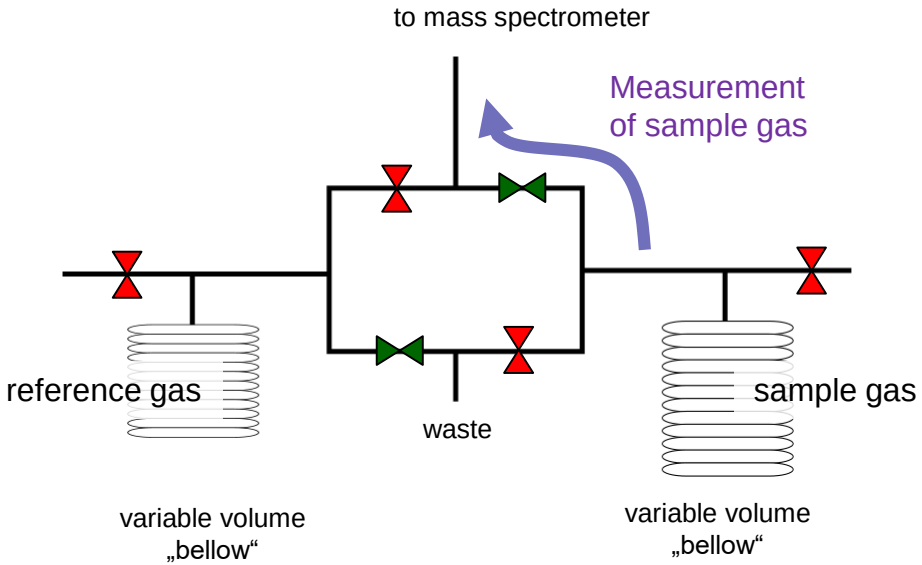
    The resonance wavelengths differ for different isotopes (of one element)
  - The extent of absorbtion depends on the concentration of the isotopes. From the ratio of the strength of absorption, the isotope ratio is calculated
  - To achieve a sufficient sensitifiy and accuracy, the infrared beam travels throught the cavity very often (100 000 times)
  - The isotope concentrations (and ratios) can be calculated from the decay of the beam caused by abosrption

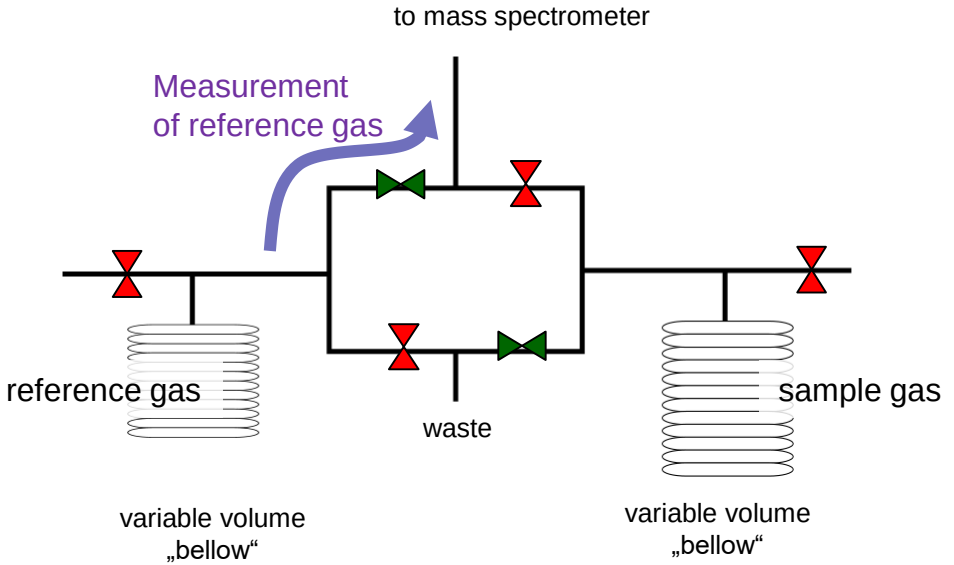




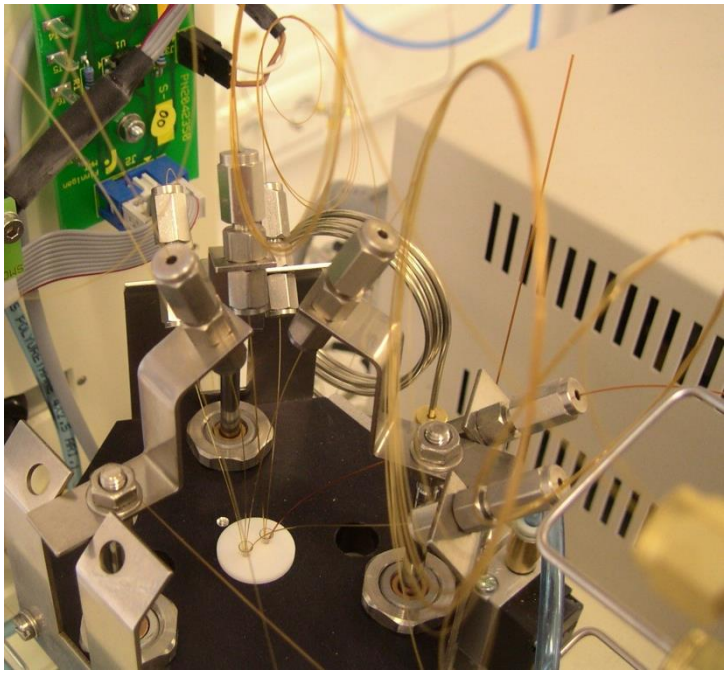
Peripheral devices

- Dual-Inlet (DI-IRMS)
  - Sample gas is introduced directly into the ion source (i.e. no carrier gas involved)
  - Reference and sample gas are measured alternately
    - measurement precision is very high
    - Relatively high amount of sample gas needed
    - sample preparation has to be carried out beforehand (off-line) which can be cumbersome and time consuming

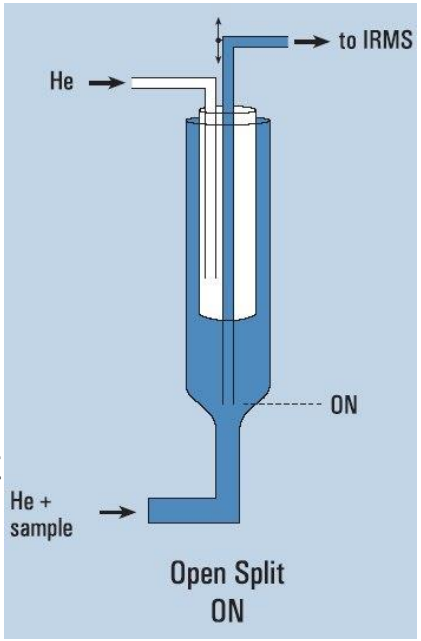


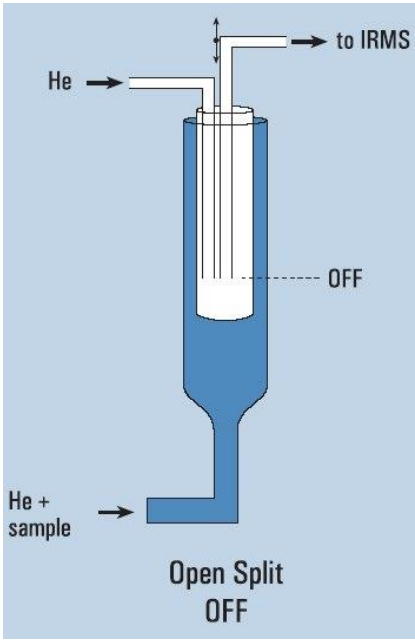


Peripheral devices



- Continuous Flow (CF-IRMS)
  - Sample gas is introduced into the ion source in a carrier gas stream *via* an **open split**
  - The amount of sample gas needed is very low (only a sall protion of the sample gas enters the ms in the open split
    → Accuracy is lower than in dual-inlet since every sample can be measured only once and the (relatively) high amount of sample gas may impede measurement
  - However sample preparation can be performed on-line which is more accurate, faster and thus allows a higher sample throughput





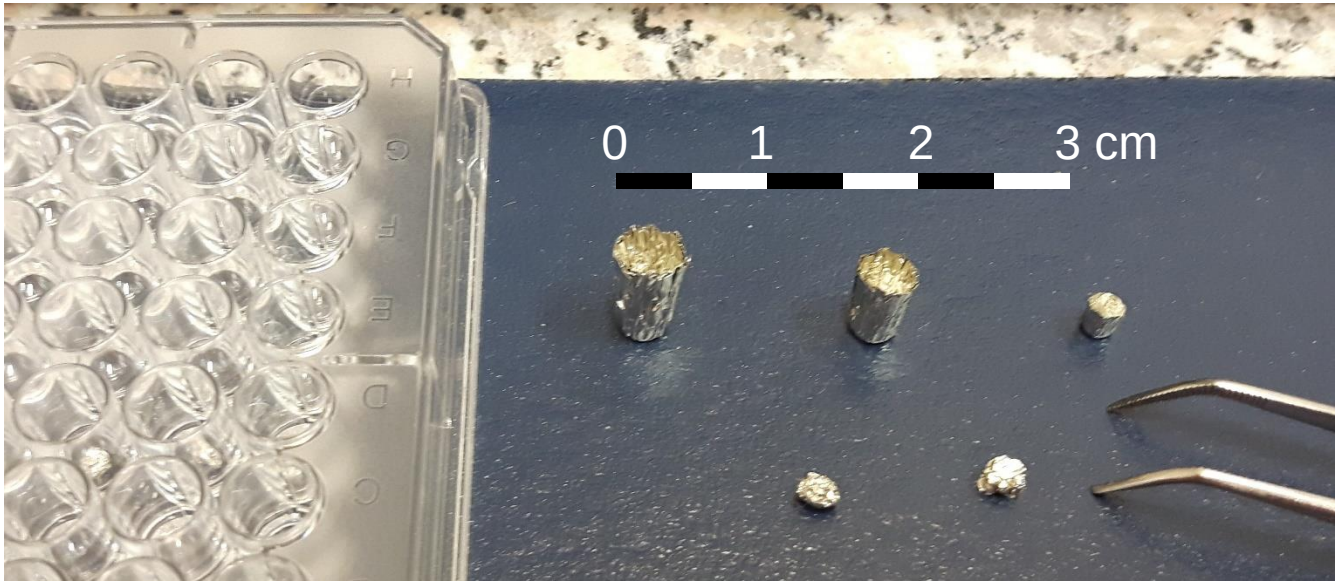
Peripheral devices

- Dual inlet (DI)
  - HDO equilibrator, H-Device (water)
  - Kiel-device (for carbonates)

- continuous flow (CF)
  - Elemental Analyser (EA-IRMS)
  - High Temperature Conversion/ Elemental Analyser (TC/EA-IRMS)
  - Gas chromatography (GC-IRMS)
  - Precon, Gasbench
  - HPLC

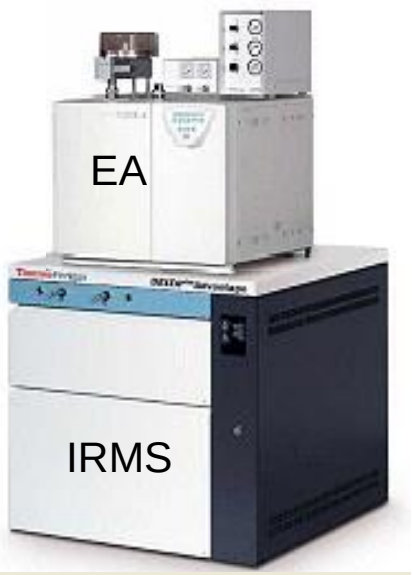
Peripheral devices

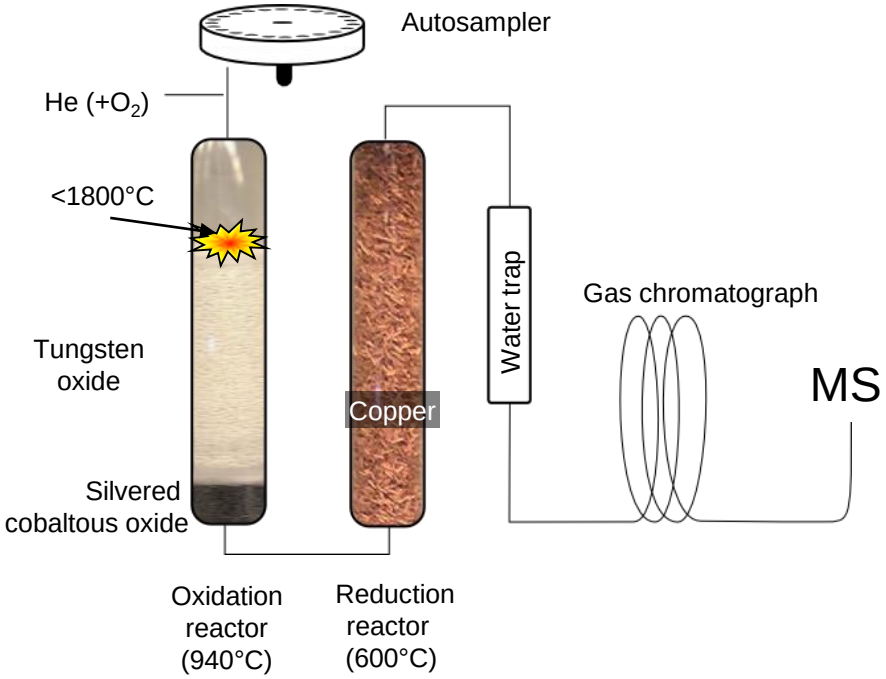
- Elemental analyser (C, N, S) – EA
  - Sample preparation
    - A representative sub sample is weighed into tin cups and rolled to a ball. Flat samples often get stuck in the autosampler.
    - Ideally, sample weight should range between 50 - 100µg N and ≤1000µg C, if necessary, 2 – 2000µg C bzw. 2 – „∞“ µg N can be analysed.
    - If C and N content is to be analysed as well, samples must be dry.

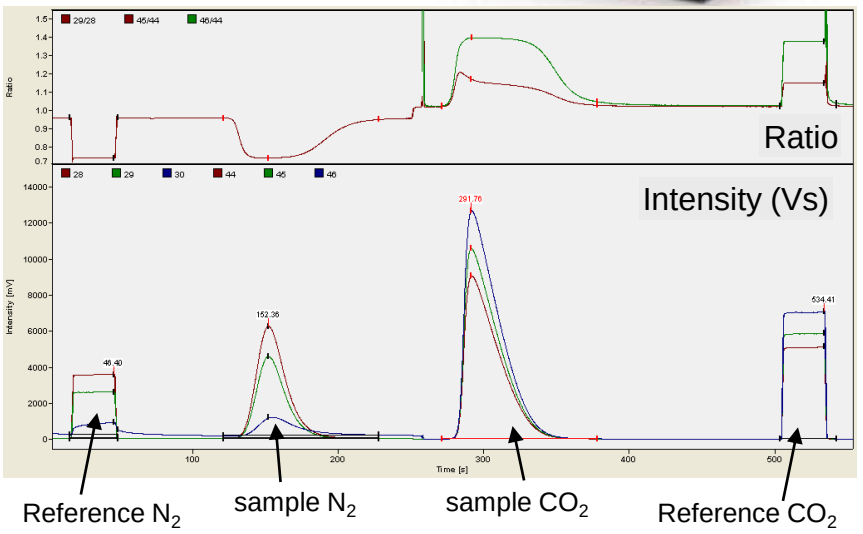


Peripheral devices

- Elemental analyser (C, N, S) – EA
  - The sample is converted to CO_2, SO_2 and NO_x by flash combustion in the oxidation reactor with O_2 added, catalysed by silvered cobaltous oxide and tungsten oxide.
  - The N containing combustion gases (NO_x) are reduced to N_2 in the reduction reactor (600°C) with copper as catalyst.
  - Sample gases pass a water trap, are separated by GC and analysed in the mass spectrometer

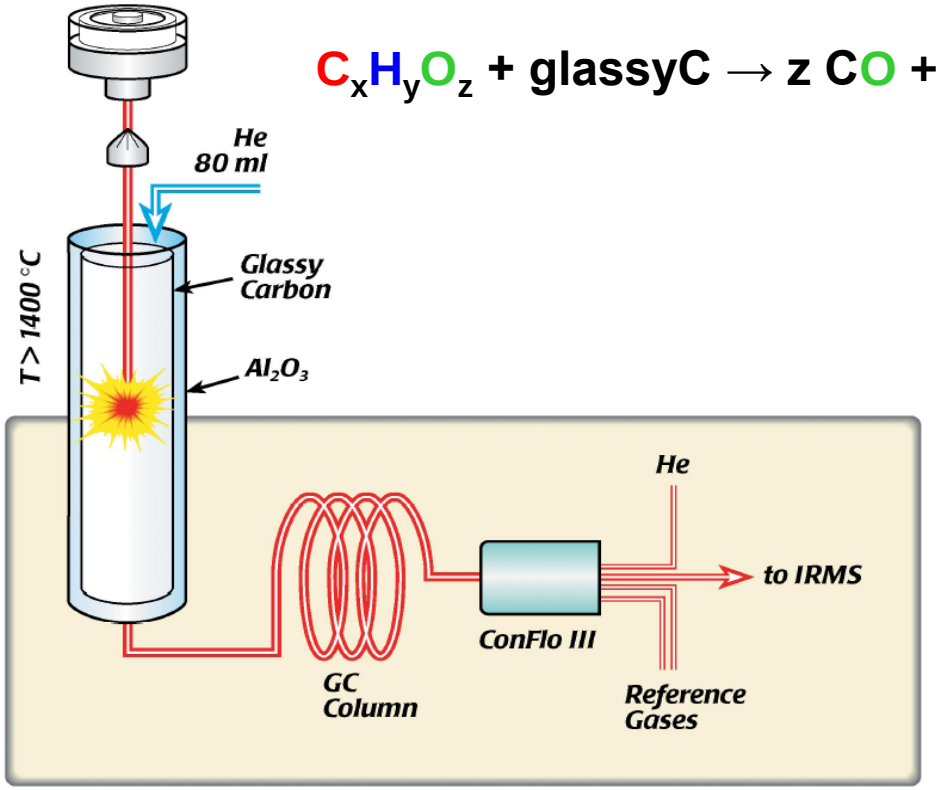






Peripheral devices

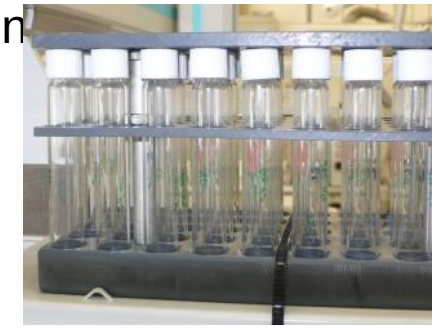
- High Temperature Conversion (O, H, NO_3-N) – TC/EA
  - Samples are pyrolysed, i.e. the oxygen of the sample is converted to CO on „glassy carbon“ with no oxygen addition, hydrogen is converted to H_2, Nitrate-N to N_2

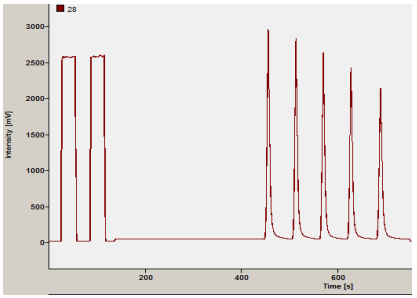


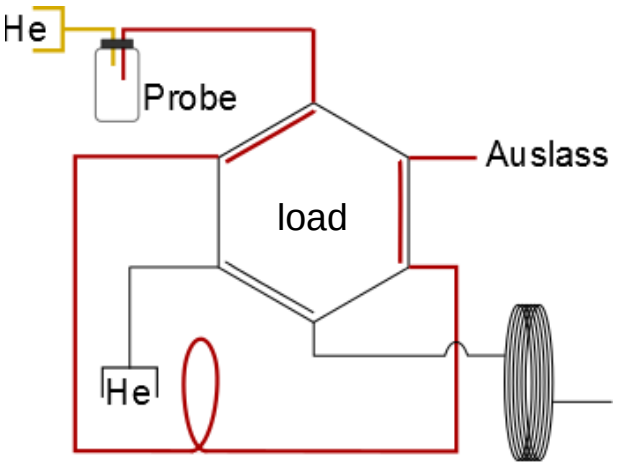
$$C_xH_yO_z + glassyC \rightarrow z\ CO + y/2H_2 + x\text{-}z\ C$$

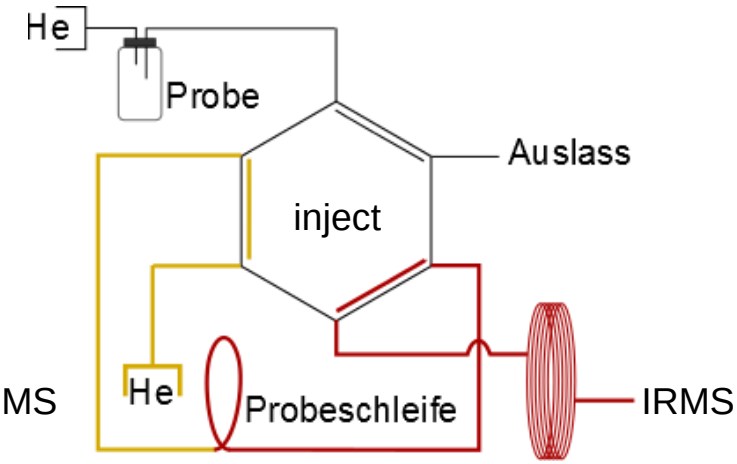
Peripheral devices

- Gas samples
  - Gas samples with high enough concentration of sample (CO_2, CH_4, N_2O, N_2) are introduced directly into the mass spectrometer after water trap and gas chromatography (and conversion in sample gas if necessary)
    - Direct injection: Sample is injected with a syringe onto the chromatographic colum via a septum
    - Loop-injection: Sample is flushed into a sample loop with a stream of He. The sample loop is then directed into the mass sectrometer in a He stream
      - Sample gas will not be contaminated by atmospheric compounds



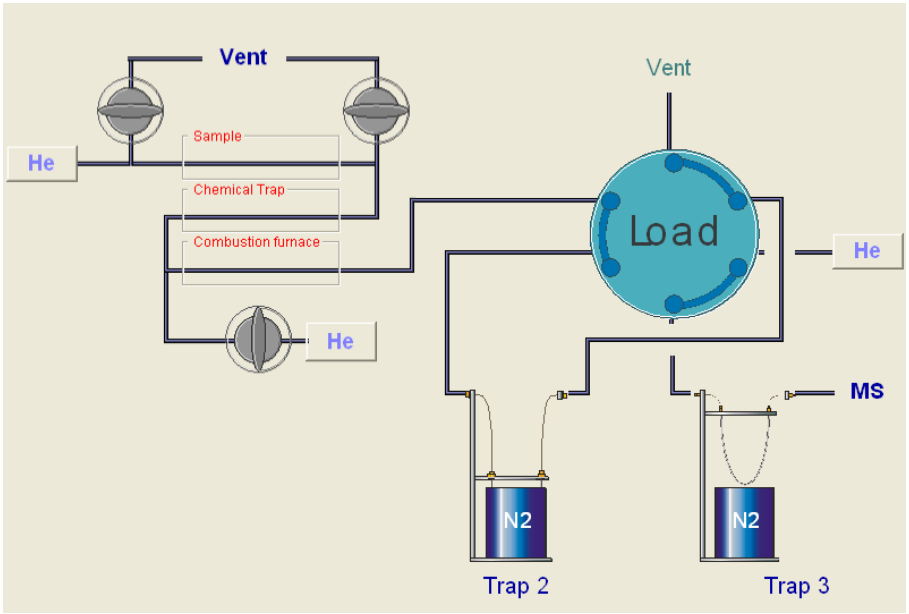


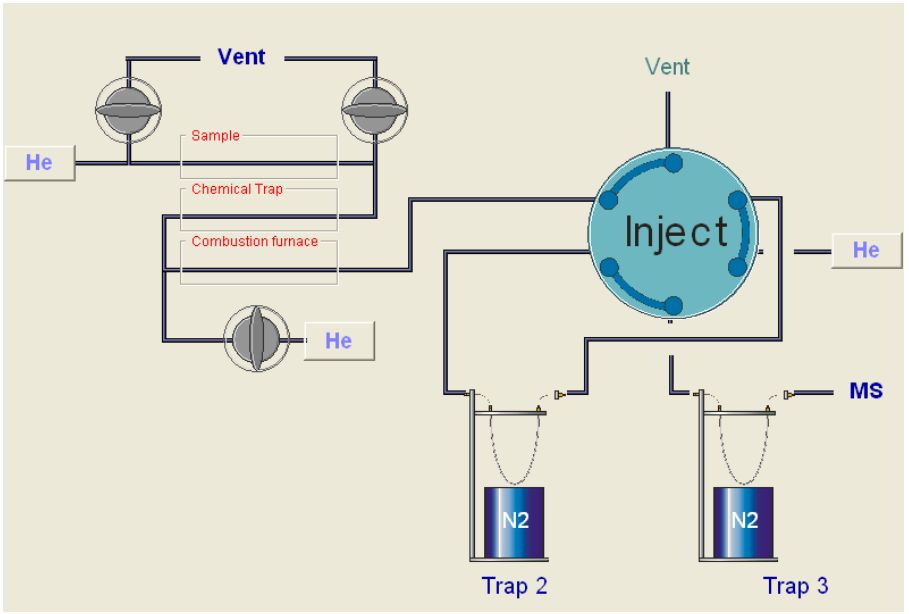




Peripheral devices

- Gas samples
  - Gas samples with high enough concentration of sample (CO_2, CH_4, N_2O, N_2) are introduced directly into the mass spectrometer after water trap and gas chromatography (and conversion into sample gas if necessary)
  - Gas samples with small concentrations are frozen in liquid nitrogen to accumulate sample (cryo focus)
    → **Precon**





Peripheral devices

- Water
  - Determination of H and O isotopes in one sample is possible via TC/EA with high sample throughput
  - Liquid water can be equilibrated with CO_2 in gas phase. Once oxygen exchange between CO_2 and H_2O reaches equilibrium, the resulting CO_2 gas can be analysed and O isotopic composition of the sample can be calculated.

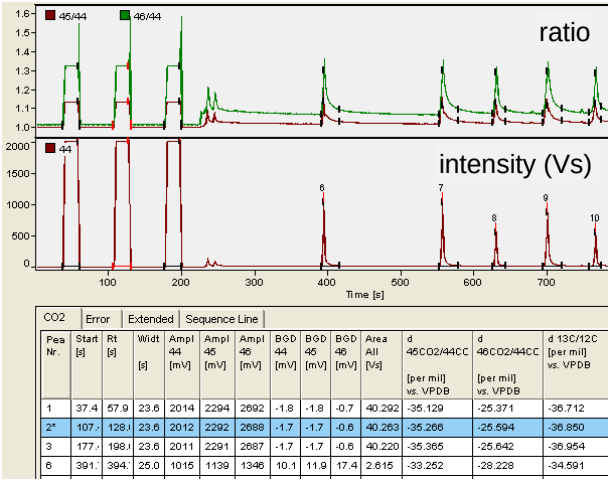
$$O{=}C{=}O + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons O{=}C{=}O + H_2O$$

    - O exchange is subject to fractionation. This fractionation is temperature dependent and thus the reaction temperature needs to be precisely controlled.
  - Water can be reduced on a chromium reactor to H_2 (H/Device)

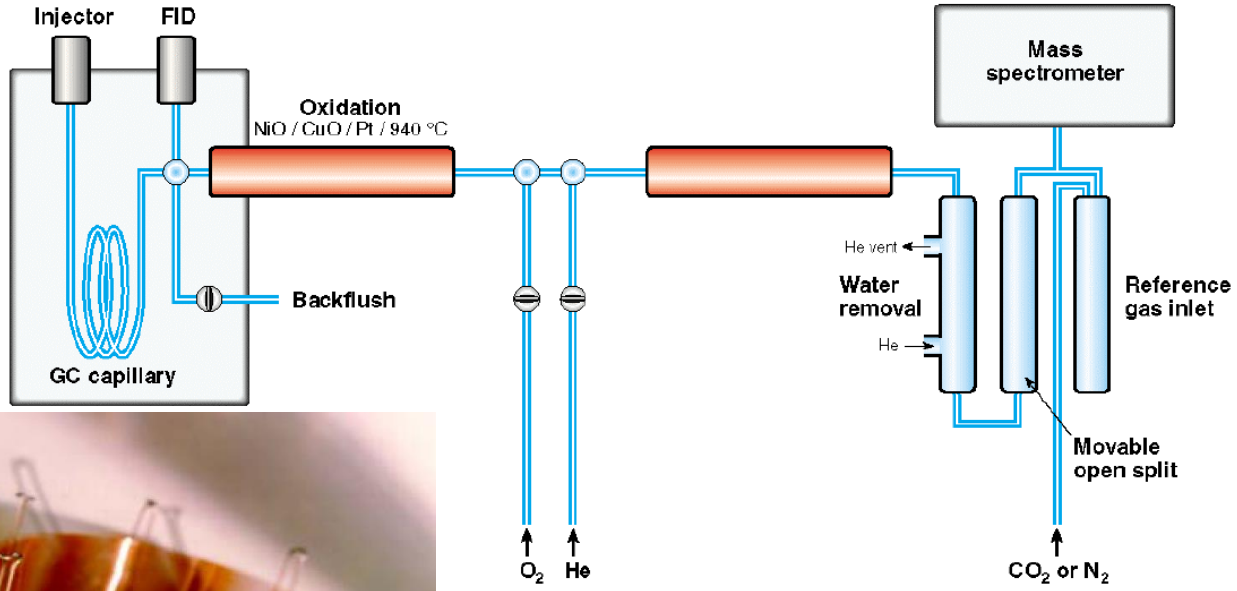
$$2\,Cr + 3\,H_2O \rightleftharpoons Cr_2O_3 + 3\,H_2$$

Compound specific isotope analysis (CSIA)

- GC-C-IRMS (gas chromatography-combustion-IRMS)
  - Separation of sample compounds by gas chromatography with subsequent
    - conversion to **C**O_2, **N**$_2$ (EA-mode)

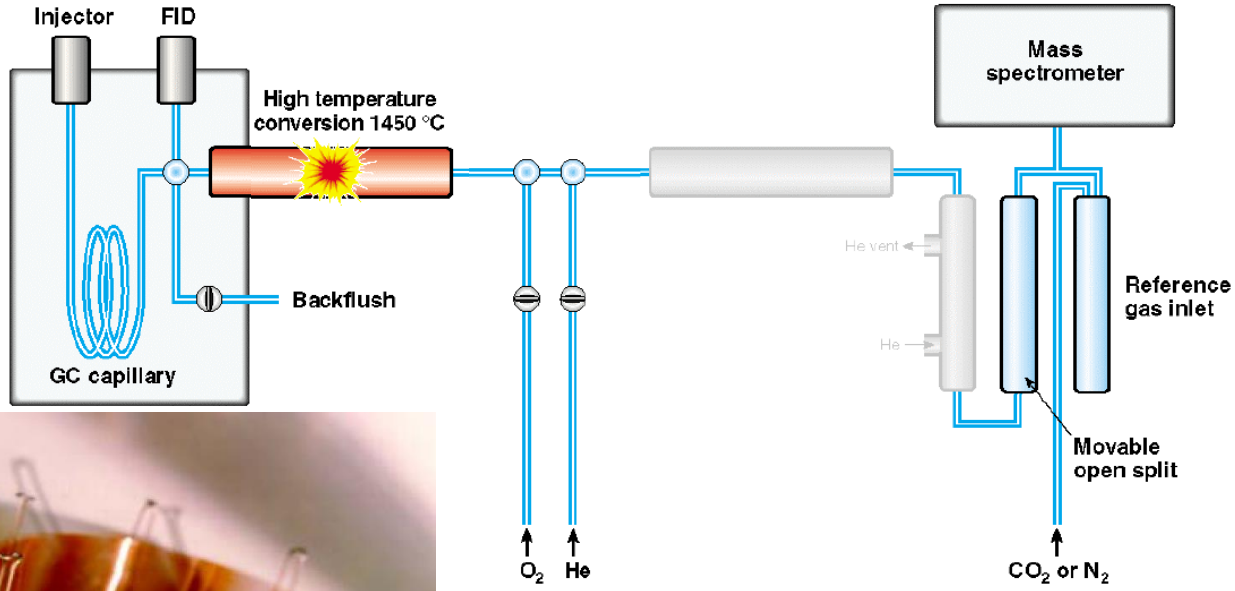


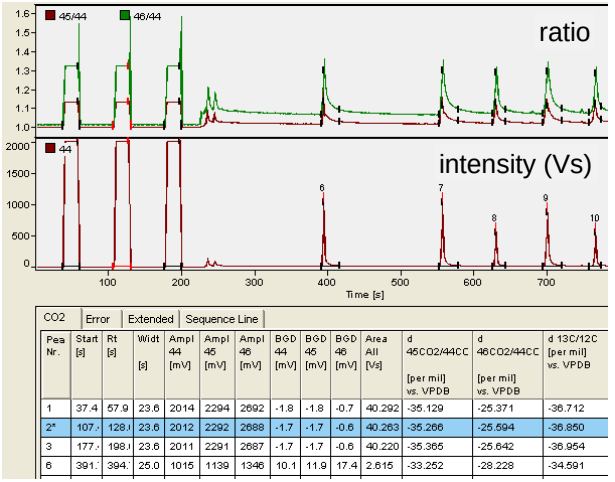
Pea Nr.	Start [s]	Rt [s]	Widt [s]	Ampl 44 [mV]	Ampl 45 [mV]	Ampl 46 [mV]	BGD 44 [mV]	BGD 45 [mV]	BGD 46 [mV]	Area All [Vs]	d 45CO2/44CC [per mil] vs. VPDB	d 46CO2/44CC [per mil] vs. VPDB	d 13C/12C [per mil] vs. VPDB
1	37.4	57.9	23.6	2014	2294	2692	-1.8	-1.8	-0.7	40.292	-35.129	-25.371	-36.712
2*	107.4	128.0	23.6	2012	2292	2688	-1.7	-1.7	-0.6	40.263	-35.266	-25.594	-36.850
3	177.4	198.0	23.6	2011	2291	2687	-1.7	-1.7	-0.6	40.220	-35.365	-25.642	-36.954
6	391.1	394.1	25.0	1015	1139	1346	10.1	11.9	17.4	2.615	-33.252	-28.228	-34.591



Compound specific isotope analysis (CSIA)

- GC-C-IRMS (gas chromatography-combustion-IRMS)
  - Separation of sample compounds by gas chromatography with subsequent
    - conversion to CO, H_2 (TC/EA-mode)

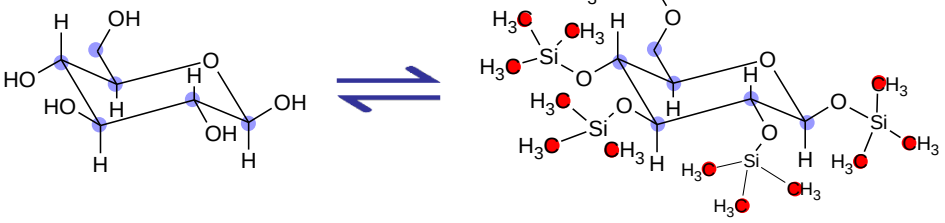




Pea Nr.	Start [s]	Rt [s]	Widt [s]	Ampl 44 [mV]	Ampl 45 [mV]	Ampl 46 [mV]	BGD 44 [mV]	BGD 45 [mV]	BGD 46 [mV]	Area All [Vs]	d 45CO2/44CC [per mil] vs. VPDB	d 46CO2/44CC [per mil] vs. VPDB	d 13C/12C [per mil] vs. VPDB
1	37.4	57.9	23.6	2014	2294	2692	-1.8	-1.8	-0.7	40.292	-35.129	-25.371	-36.712
2*	107.4	128.0	23.6	2012	2292	2688	-1.7	-1.7	-0.6	40.263	-35.266	-25.594	-36.850
3	177.4	198.0	23.6	2011	2291	2687	-1.7	-1.7	-0.6	40.220	-35.365	-25.642	-36.954
6	391.1	394.1	25.0	1015	1139	1346	10.1	11.9	17.4	2.615	-33.252	-28.228	-34.591

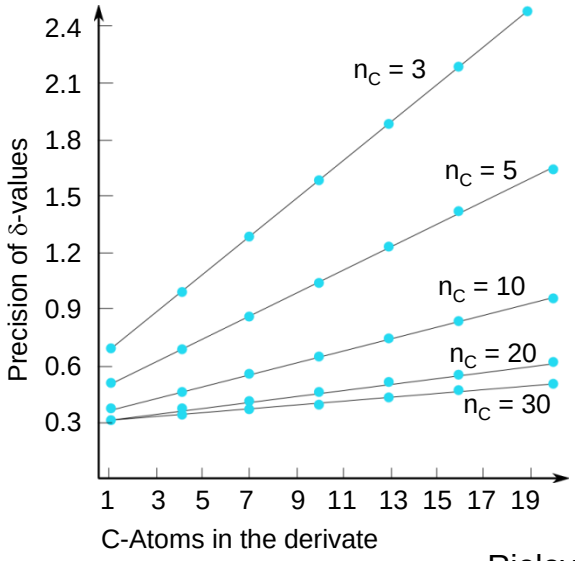
Compound specific isotope analysis (CSIA)

- Derivatisation before GC separation
  - Most polar molecules (sugars, amino acids, …) cannot be separated by GC because they are not volatile
  - Polar moieties must be converted into non polar moieties to make molecules volatile
  - Problems
    - The derivatisation reaction may be subject to fractionation (which can be accounted for if fractionation is reproducible but still reduces accuracy)
    - The introduction of additional C atoms into the target compound will decrease accuracy.
      - This effect will increase with increasing ratio $C_{sample}/C_{derviatisation\ reagent}$



6 C-Atoms

6 + 18 C-Atoms



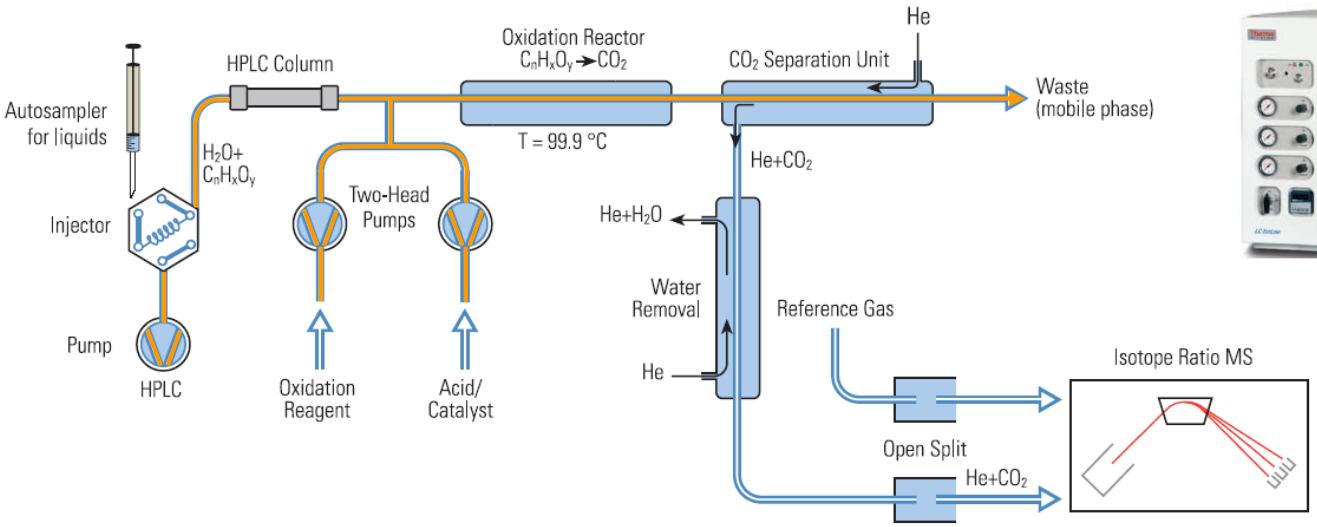
Rieley, 1994

Compound specific isotope analysis (CSIA)

- Liquid chromatography (LC-IRMS)
  - Suitable for polar (i.e. water soluble) non volatile (i.e. not accessible via GC) substances (sugars, amino acids,…)
  - Wet oxidation of sample compounds to CO_2 with peroxodisulfate (oxidant) and phosphoric acid after HPLC-separation

$$6\ S_2O_8^{2-} + C_2H_5OH + 3\ H_2O \rightleftharpoons 12\ SO_4^{2-} + 2\ CO_2 + 12\ H^+$$

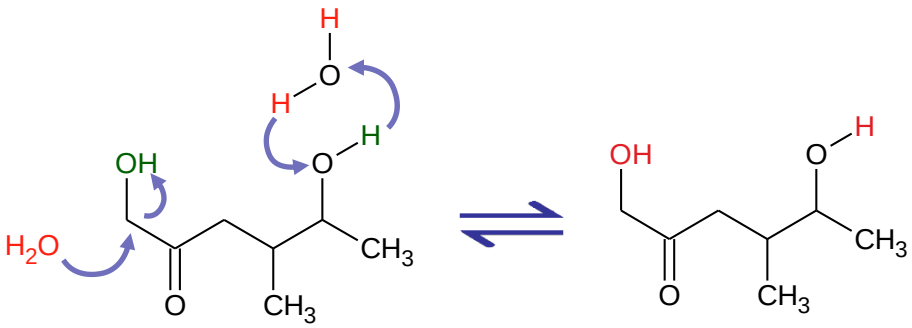
  - Transfer of CO_2 into the gas phase *via* a gas exchange membrane and measurement in the mass spectrometer



Sample prepapation – solids

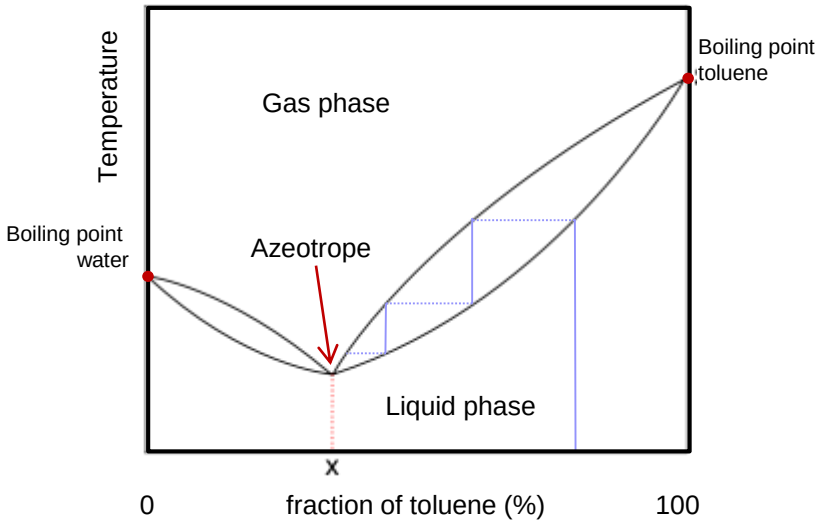
- C/N-Analysis
  - Samples must be dry and homogenous (milled)
    - See “Accuray”

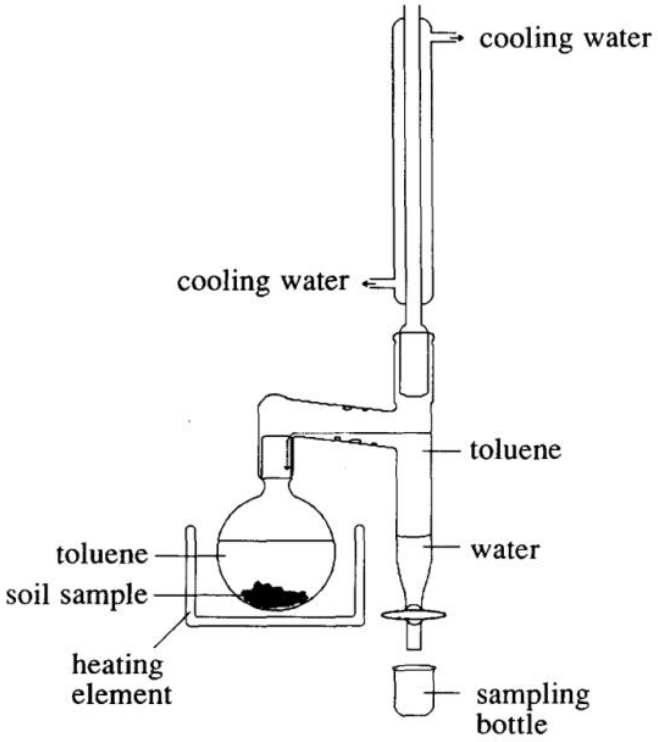
- O/H-Analysis
  - Samples must be dry and homogenous (milled)
    - See “Accuray”
  - Samples may exchange O and H with atmospheric water vapour, this effect must be corrected for
    - One way to do so is to equilibrate samples with water vapour of known isotopic composition prior to measurement
    - The isotopic composition of samples can only be measured on molecules that contain at least some irreversibly bound O- and H-atoms



Sample Preparation - Water

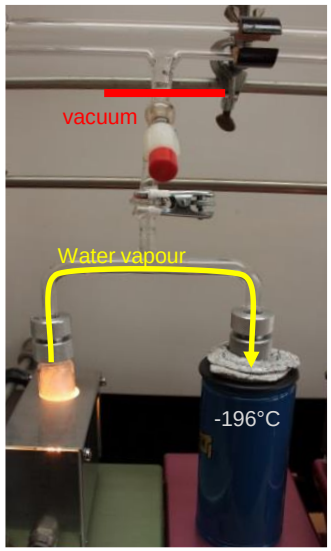
- Centrifugation
- Azeotrope distillation
  - Excess toluene is added to the sample. Toluene and water form an azeotrope and thus all water is removed from the sample when toluene is evaporated.
  - Toluen and water are (almost) inmiscible. Therefore a two phase system is formed after evaporation
    and water can easily be separated from the toluene.





Sample Preparation - Water

- Cryo-distillation
  - The sample is frozen in liquid nitrogen (-196°C) and the volume is evacuated
  - The sample is heated in stationary vaccum. The water vapour is condensed in the recieving flask in liquid nitrogen; the water is thus removed from the sample (almost?) completely.

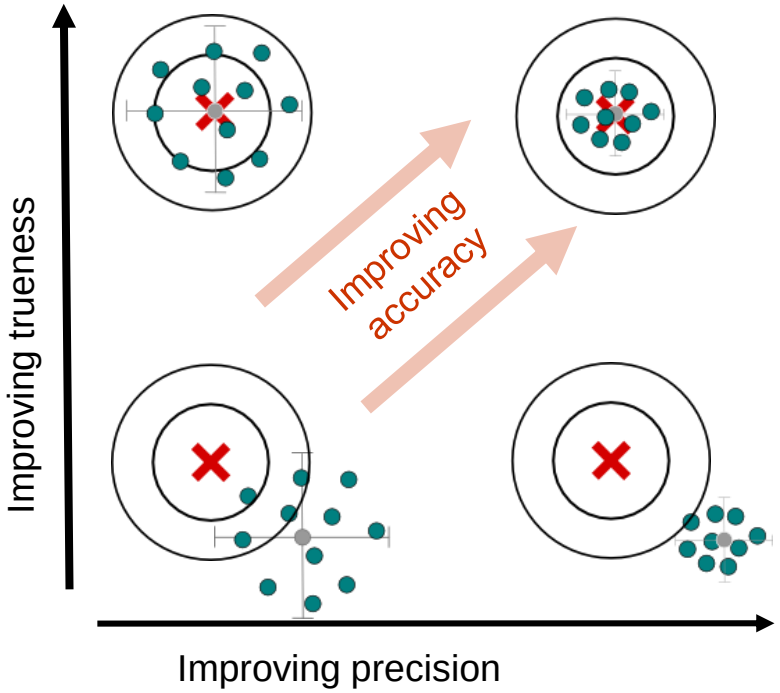


Sample preparation

- Two thing are necessary to get an ‘accurate’ result of a sample measurement
  - The value must be ‘precise’:
    - Repeated measurements give a similar result

      → The measurements have a small random error
  - The value must bee ‘true’:
    - The resulting value must be close to the ‘true’ value

      → The measurements have a small systematic error

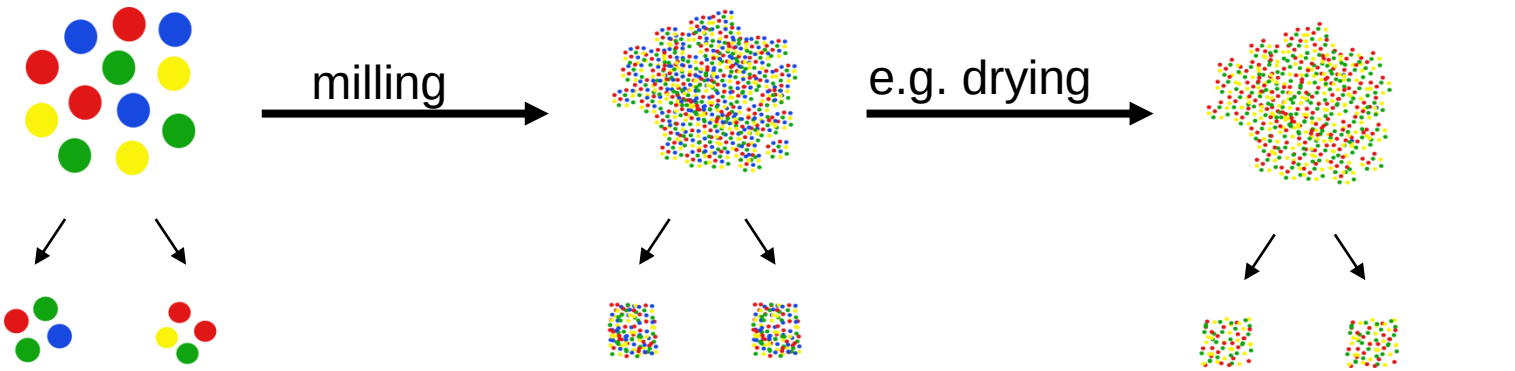


- Random errors can be identified and corrected for by repeated analysis; systematic errors are not easily detectable and therefore very malicious

Sample preparation

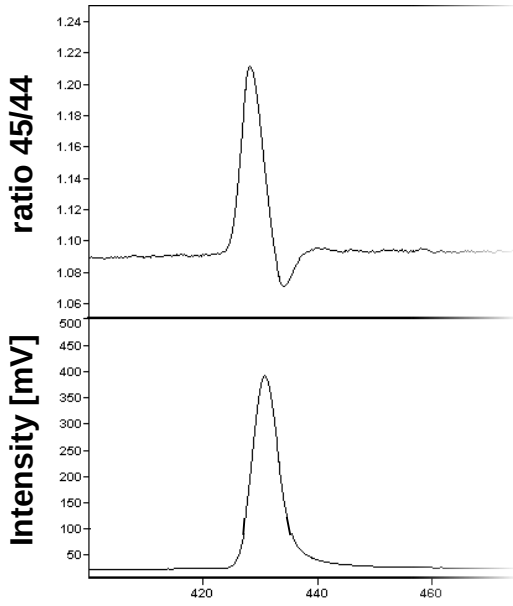
- Systematic and random errors must be avoided to get accurate results
  - Sampling: a representative subsample must be taken.

    Prerequisite is a thorough homogenisation of the sample (milling)
    - Alternatively, the whole sample can be analysed (e.g. whole animals, buds, …)
  - Systematic loss of parts of the sample must be avoided (e.g. by evaporation during drying) because this might alter the isotopic composition of the bulk sample.



Sample preparation

- Systematic and random errors must be avoided to receive accurate results
  - Reactions must be quantitatively
    (cf. Raleigh distillation: no fractionation for complete conversion)
    - Alternatively, reactions must be reproducible so that fractionation is constant and can be corrected for
  - The whole sample must be analysed
    - E.g. chromatography: heavy isotopes preferentially elute at the peak front.
      The „true“ isotopic ratio can only be measured by integrating the whole peak



Accuracy – Fractionation issues

- Fractionation or loss of isotopically distinct sample fractions during sample preparation may lead to biased results
  - Loss of specific sample fractions may be due to
    - Drying of samples (loss of volatile compounds)
    - Incomplete sample recovery (e.g. fatty components stick to the mortar when grinding samples)
  - The inaccuracy produced by specific loss increases with the isotopic difference between recovered and lost sample and amount of sample lost
    - Specific loss usually is less problematic for natural abundance samples
      - If the difference between different compartments of a tissue is 2 mUr, a mass loss of 10% loss leads to < 0.2 mUr deviation
    - Specific loss may especially affect tracer experiments
      - E.g. leaf material from a labelled CO_2 uptake experiment is washed in water
        - Soluble compounds will be lost, among this freshly assimilated labelled glucose
      - The resulting error is hard to guess, but will be very high

Accuracy – Fractionation issues

- Fractionation or loss of isotopically distinct sample fractions during sample preparation may lead to biased results
  - Fractionation may occur due to
    - Chromatography
    - Incomplete extraction of target material from the sample (i.e. precipitation of carbonate to extract dissolved CO_2 from liquid solutions)
    - …
  - The inaccuracy produced by fractionation during sample preparation increases with
    - Fractionation factor
    - Importance (yield) of the fractionating process
    - The inaccuracy can be calculated from

$$\delta_{Substrate} - \delta_{0-Substrate} \cong \varepsilon \ln f$$

      - e.g. CO_2 loss during trapping of CO_2 as carbonate
        ε = 10 mUr; 2% loss → Δ = 0.8 mUr
        5% loss → Δ = 1.6 mUr

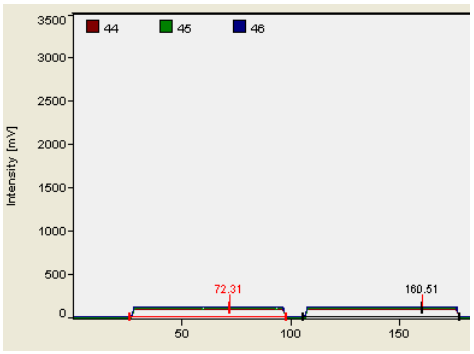
Accuracy – Contamination

- Contamination
  - Contribution on non-sample material to the analyte
    - Measurement blank (see e.g. EA-measurements)
    - Carry-over from previous sample, during measurement or sample preparation
  - Contamination is especially important for labelled samples
    - e.g. carry-over in EA-measurements
      - Carry-over from one labelled sample (8 at% ^{15}N) leads to an error of 20 mUr in the tenth sample (carry-over amount of 0.1%)
    - Carry-over can also occur during sample preparation, e.g. using the same mill for labelled and non-labelled samples

Accuray

- Random and systematic errors must be avoided to get accurate results
  - Short, high peak yield more accurate results than long, flat peaks
    - The reason for this is the background value that is substracted over the total peak width. The longer the peak, the higher the influence of the (more or less accurate) background determination

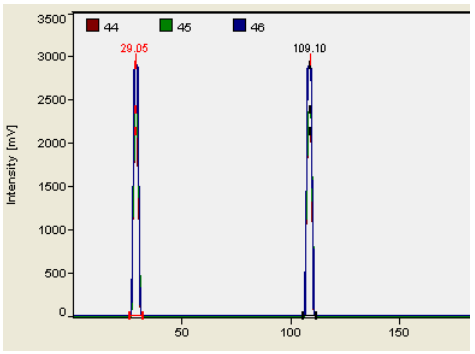
Long, flat peaks



Standard deviation = 0,06

Short, high peaks

Standard deviation = 0,16

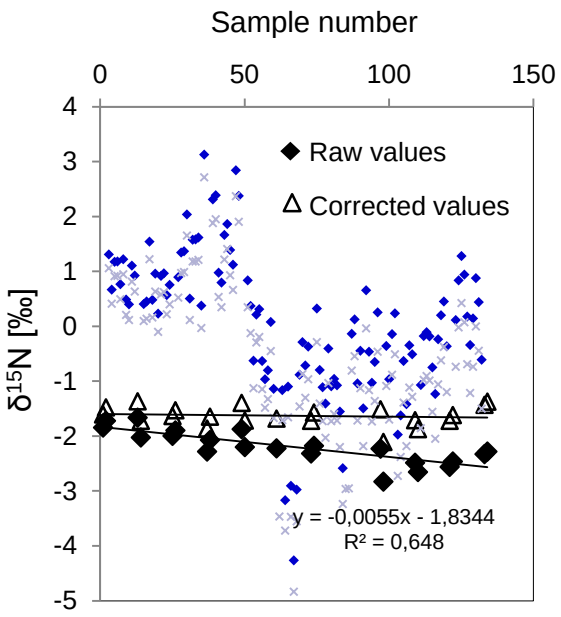


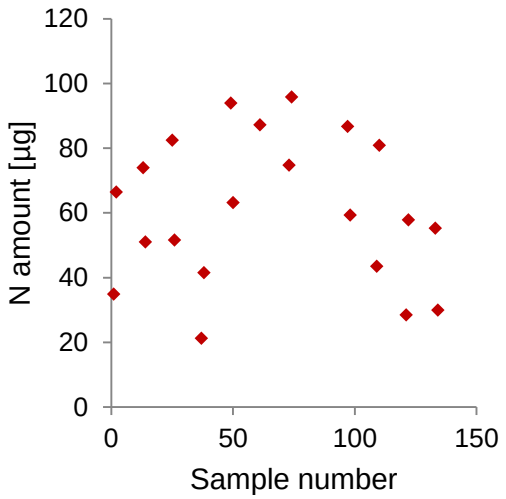
Accuray – Post hoc correction

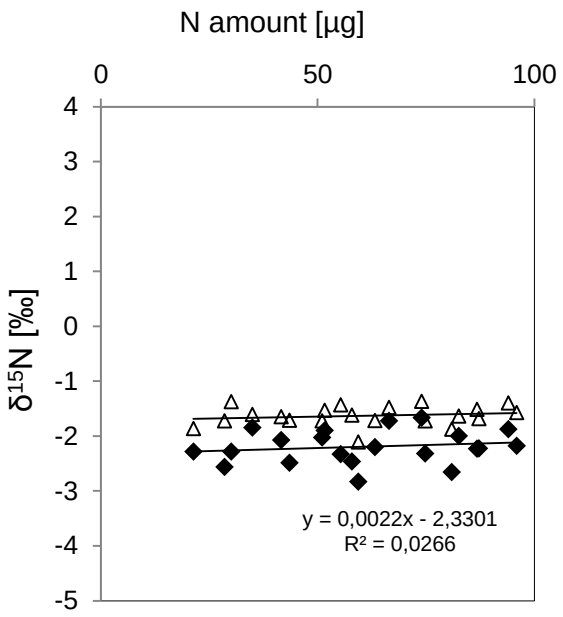
- The samples are measured relative to a reference gas (which is measured directly without sample preparation, reaction, …) and anchored on the international scale with lab standards.
- Lab standards are (also) used to determine and correct for machine drift (in the peripheral devices or the mass spec itself)
  - Time drift: The delta values of the lab standards vary within the sequence (caused e.g. by temperature drift, ???,???)
  - Amount drift: The delta value changes with changing sample amount (i.e. peak height), caused by e.g. impurities in the ion source, ???, ???, the reasons are often not clear)
  - Blank-correction: Impurities (from periphery or mass spec) can affect the delta values especially for small sample sizes
    - Blank correction is usually not necessary if a chromatographic step separates impurities from the sample

Accuray – Post hoc correction

- Drift correction
  - The results of the standard samples are used to check for time or quantity drift
    - Caution: Big and small sample sizes must be distributed eavenly over the measurement sequence to allow the distiction between time or quantity drift
  - Standards samples are corrected to the true value
  - The same correction is applied to all unknown samples

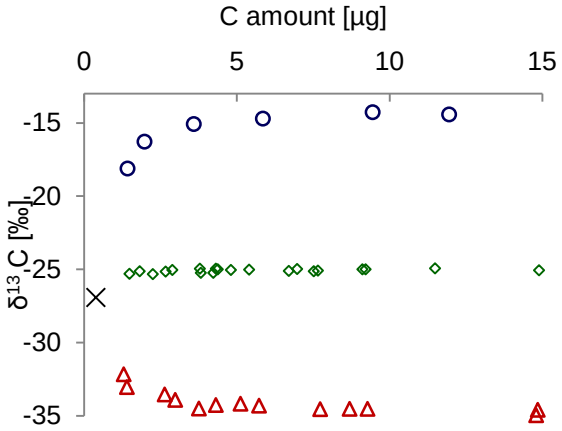


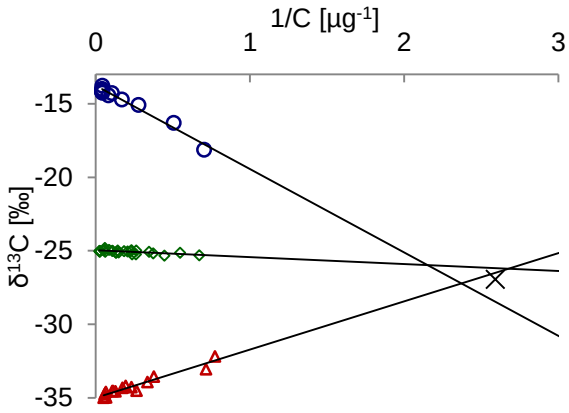


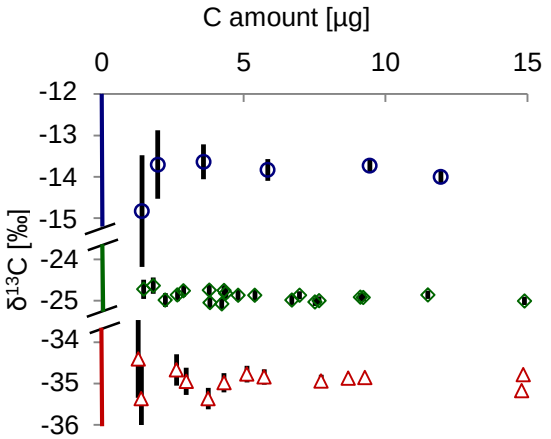


Accuray – Post hoc correction

- Blank correction
  - Contributions of non-sample material (e.g. air-N2, carbon from tin cups for EA analysis,…) must be substracted from the Resuls of the measurements
  - The amount and isotopic composition of the blank can be determined by
    - direct measurement (if blank is high enough for direct isotopic analysis)
    - extrapolation of the plot 1/amount vs. delta value (Keeling-plot) of several standars substances of differing isotopic composition
  - Mathematical correction of the measured values increases measurement uncertainties substantially because of error propagation (especially if sample and blank have very different isotopic composition)



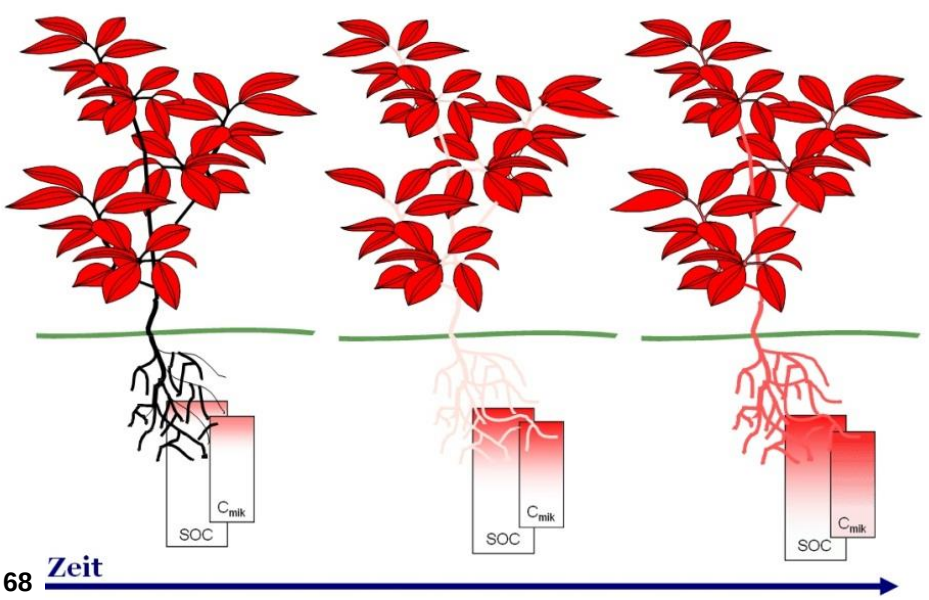


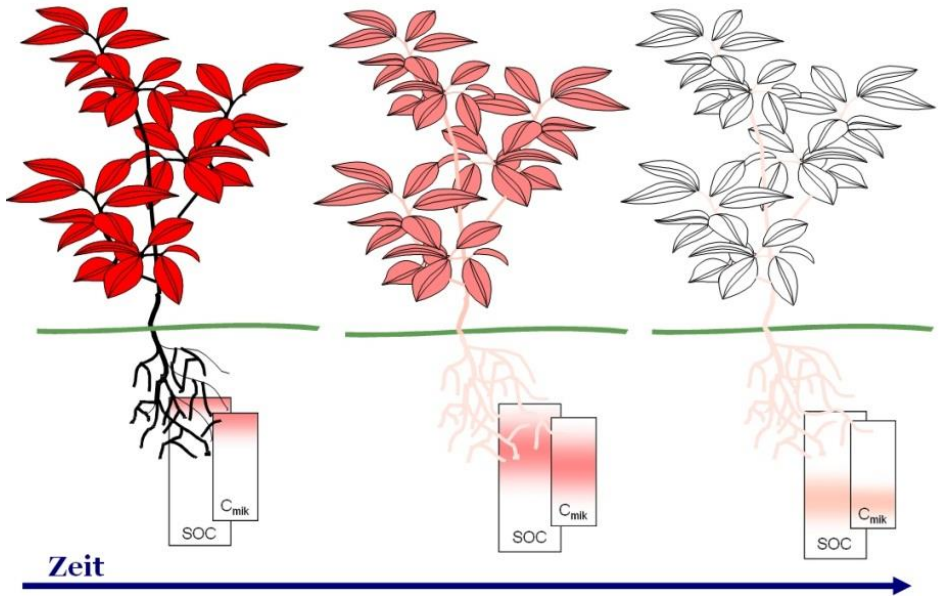


Labelling techniques

- Long term labelling (**Continuous labelling**)
  - Suitable to quantitatively determine (net) turnover rates
    - E.g. trees growing under CO_2-labelled atmosphere

- Short term labelling (**Pulse-chase labelling**)
  - Suitable to determine the fate of a molecule (or some atoms) within an ecosystem
  - Suitable to elucidate processes that usually are not detectable (due to restricted time or amount)
    - E. g. labelled litter is decomposed in soil monoliths

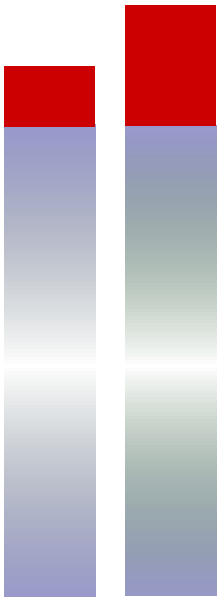




Labelling techniques

- Methods of tracer application
  - High enrichment to minimise disturbance, fractionation becomes negligible
    - Small amount of added substances, e.g. $^{15}NO_3^-$ (99at%) do not disturb nitrogen cycling in the soil but allow recovery of ^{15}N in all soil compartments (nitrate, ammonium, dissolved organic nitrogen, organic nitrogen, plant, N_2O, N_2)
  - Low enrichment (within natural abundance) to minimise cost
    - Substances from natural sources can be added as a tracer to a system with different isotopic composition
      - CO_2 from fossil methane ($\delta^{13}C$ = -48 ‰ *vs.* -8 ‰ in the atmosphere)
      - C_4 plants (e.g. maize, $\delta^{13}C$ = -12 ‰) on a C_3 soil (e.g. forest, wheat; -30 ‰)
    - Suitable for long term experiments (traceability will increase with time), but fractionation must be accounted for

Calculations with enrichments



Atom%

B 1,122
A 1,100
N 1,078

⋮

0 atom%

B - A = 0,022%

B : A = 102 %

Atom%excess

B 0,044
A 0,022
N 0,000 atom%excess

B - A = 0,021866%

B : A = 200%

A - N = 0,021876%

Delta-permil

B 15,0
A 0
-5,0
N -25,0 permil

⋮

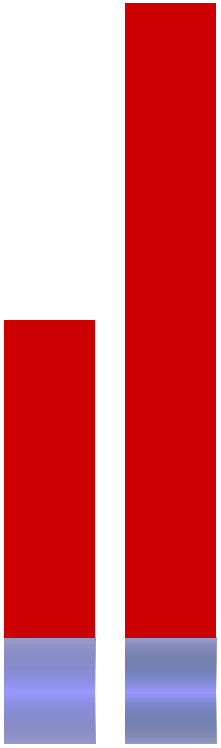
-1000

B - A = 20,0‰

B : A = -300%

A - N = 20,0‰

Calculations with enrichments



Atom%

B 9,078

A 5,078

N 1,078

0 atom%

B - A = 4,00%

B : A = 179%

Atom%excess

B 8,000

A 4,000

N 0,000 atom%excess

B - A = 4,00%

B : A = 200%

A - N = 4,00%

Delta-permil

B 7930

A 3785

N -25 0

-1000 permil

B - A = 4145‰

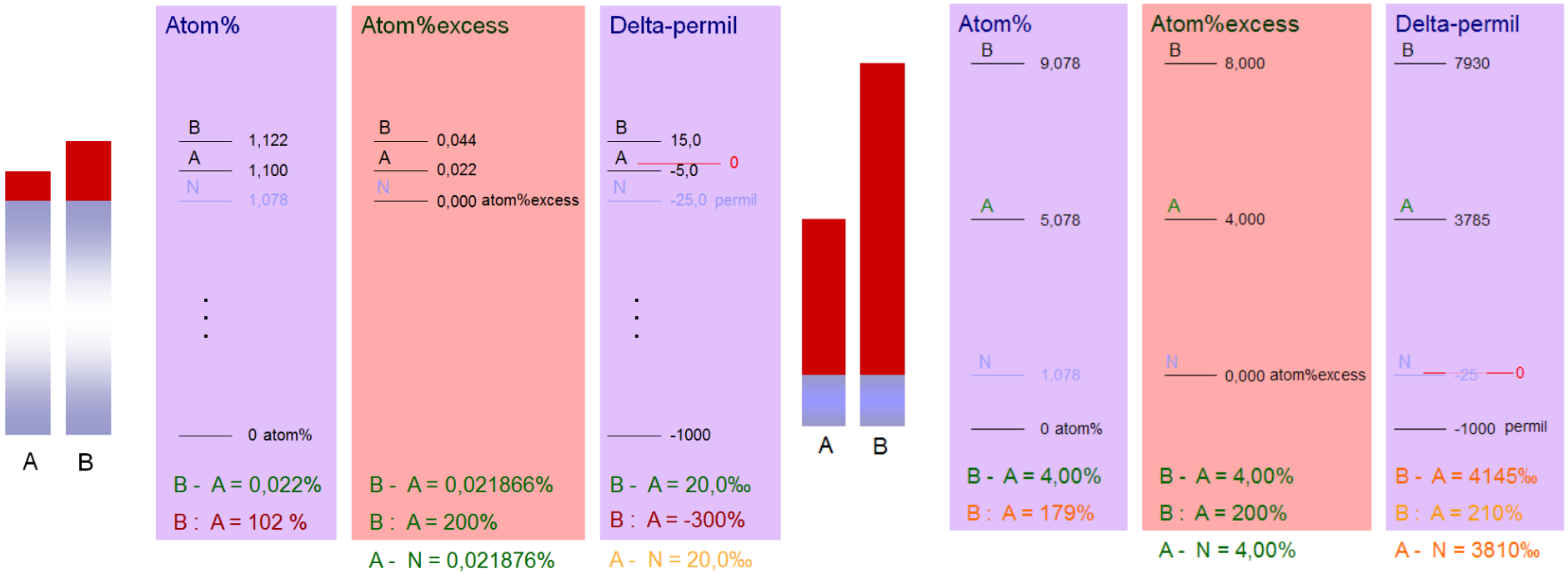
B : A = 210%

A - N = 3810‰

Calculations with enrichments

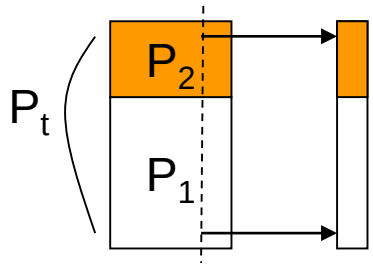
- Low enrichment
  - High influence of the background value → Atom% vs. Atom%excess

- High enrichment:
  - Small influence of the background value
  - Significant difference between atom% and delta-permil



Labelling techniques

- Isotope pool dilution
  - To determine the size of a pool, a known amount of labelled substance is added to that pool. The amount of enrichment in the total pool indicates the size of the total pool without the need to extract the complete pool.



$$P_t \cdot at\%_t = P_1 \cdot at\%_1 + P_2 \cdot at\%_2$$

and also

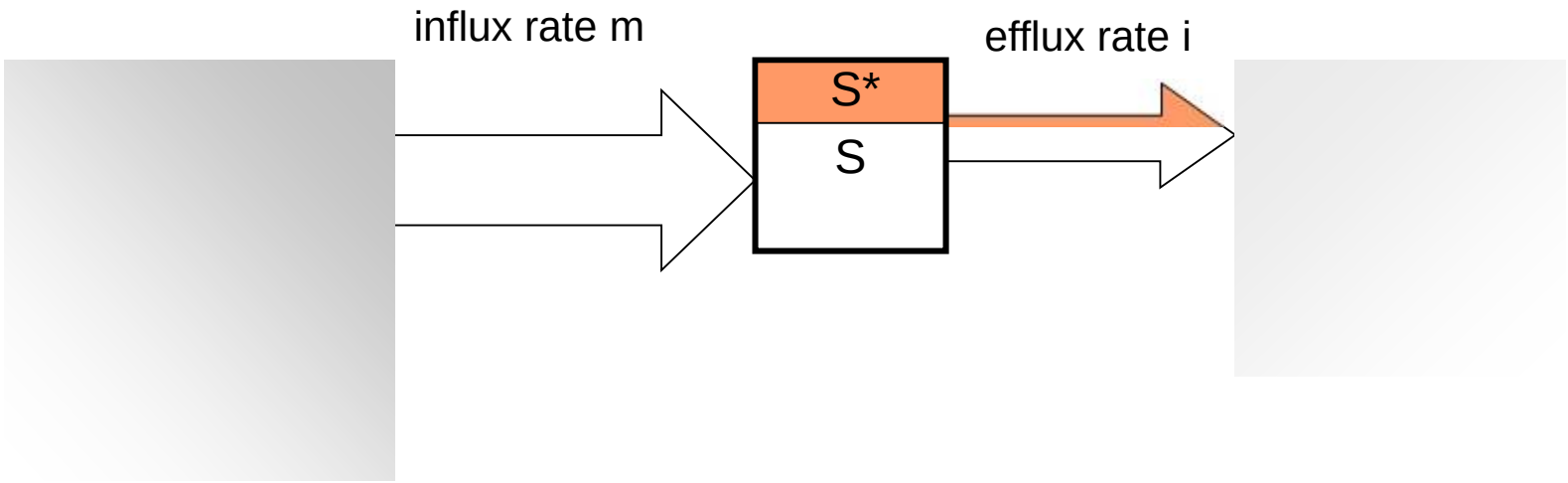
$$P_t = P_1 + P_2$$

$$\Rightarrow P_1 = \frac{P_2 \cdot at\%_2 - P_2 \cdot at\%_t}{(at\%_t - at\%_1)}$$

P: pool size
at%: enrichment of the pool

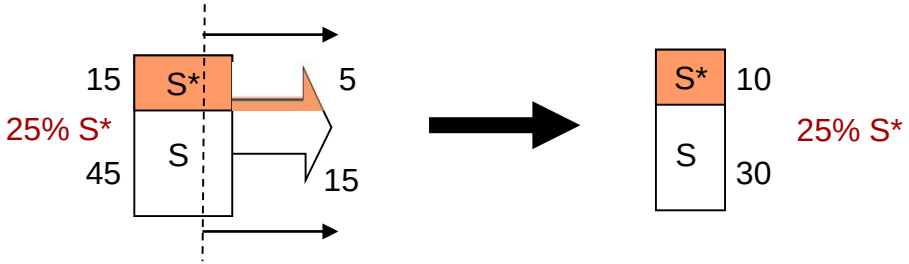
Labelling techniques

- Isotope pool dilution
  - Problem: Determination of influx and efflux of a pool
  - To determine gross turnover rates (influx rate m, efflux rate i) – as opposed to net flux rates (i.e. the change of pool size over time (=m – i) – the pool S is homogeniously isotopically labelled
  - This has the consequence that the efflux i from the pool is isotopically labelled but influx m is not.

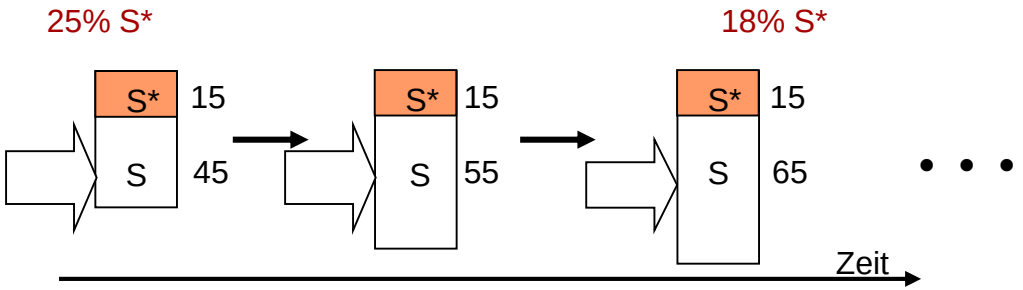


Labelling techniques

- Isotope pool dilution
  - Efflux from the pool (rate i) do not change isotopic composition of the pool, since both labelled and unlabelled substance is lost



  - Influx of new unlabelled substance (rate m) will change (dilute) isotopic composition of the pool



Labelling techniques

- Isotope pool dilution
  - **Prerequisites**
    - The examined pool is labelled homogeniously
    - All processes obey a zero order kinetic (i.e. rates are constant and independent of pool sizes)
    - Efflux and influx are fractionation free (or labelling is high enough to minimize this effect)
    - Labelled substance that leaves the pool will not enter the pool again
  - Influx rate **m** and efflux rate **i** can be calculated according to

$$m = \frac{S_0 - S}{t} \frac{\ln \frac{S_0^* S}{S^* S_0}}{\ln \frac{S_0}{S}}, \quad i \neq m \qquad i = \frac{S_0 - S}{t} \frac{\ln \frac{S_0^*}{S^*}}{\ln \frac{S_0}{S}}, \quad i \neq m$$

$$m = i = \frac{S_0}{t} \ln \frac{S_0^*}{S^*} \quad i = m$$

with S = substrate, S^* = labelled substrate,
subscripts t and 0 refer to the points in time t and t=0

(after Kirkham & Bartholomew 1954)

Two pool mixing model

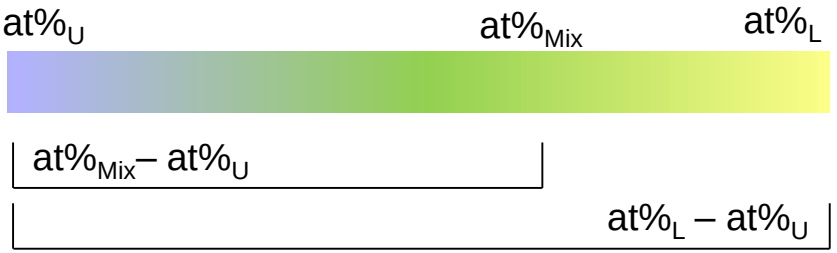
- Two pool mixing model
  - The contribution of a (labelled) part of a pool can be calculated according to:

$$M_{Mix} = M_L + M_U \qquad \text{(mass balance)}$$
$$\Rightarrow M_L = M_{Mix} - M_U$$

$$M_{Mix} \cdot at\%_{Mix} = M_L \cdot at\%_L + M_U \cdot at\%_U \qquad \text{(isotopic balance)}$$

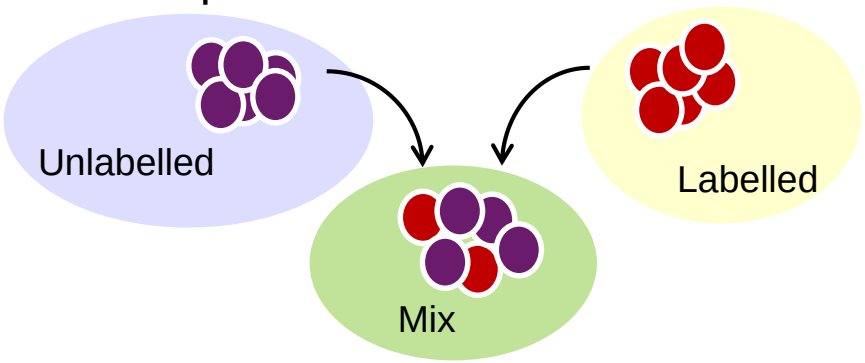
combining both formula gives

$$M_L = \left(\frac{at\%_{Mix} - at\%_U}{at\%_L - at\%_U} \right)$$



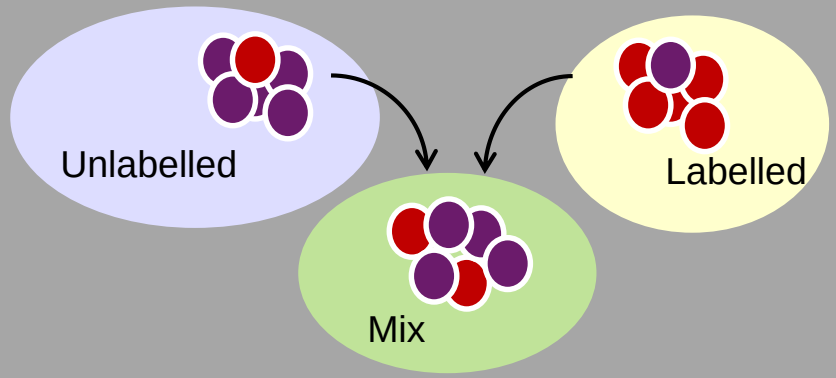
Two pool mixing model

- Example



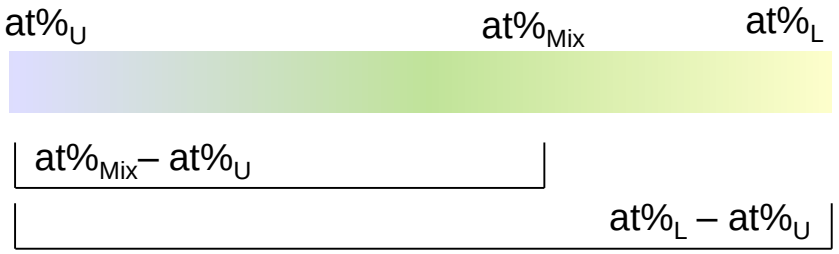
$$\text{Beitrag Labelled} = \left(\frac{at\%_{Mix} - at\%_{U}}{at\%_{L} - at\%_{U}} \right)$$

$$\text{Beitrag Labelled} = \left(\frac{\frac{2}{6} - \frac{0}{6}}{\frac{6}{6} - \frac{0}{6}} \right) = \left(\frac{2}{6} \right) = 33\%$$



$$\text{Beitrag Labelled} = \left(\frac{at\%_{Mix} - at\%_{U}}{at\%_{L} - at\%_{U}} \right)$$

$$\text{Beitrag Labelled} = \left(\frac{\frac{2}{6} - \frac{1}{6}}{\frac{5}{6} - \frac{1}{6}} \right) = 25\%$$



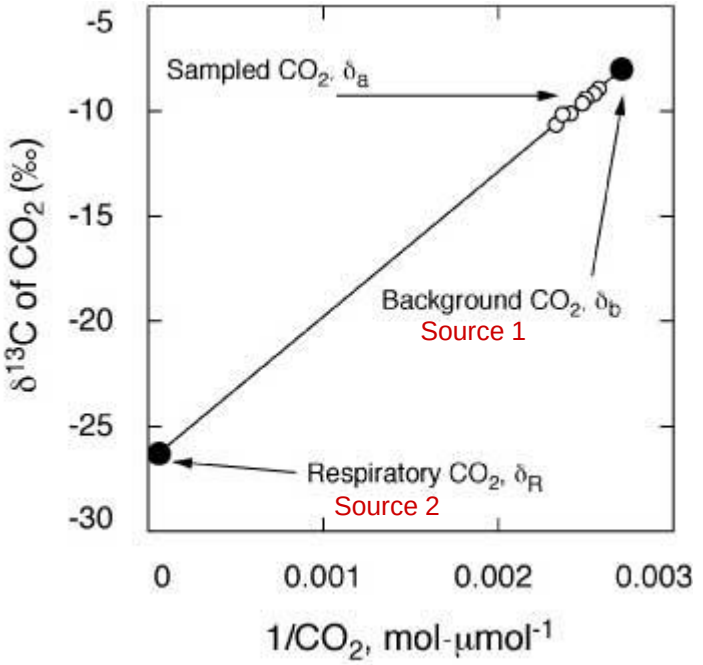
Mixing model – Keeling plot

- Analysis of a mixing system
  - Constant contribution of source 1 and varying amounts of source 2 (and: sources are isotopically different!)
    - Example: Atmospheric air (360ppm CO_2) mixed with CO_2 from soil respiration
    - The plot of the reciprocal concentration (1/c) vs. Isotopic composition results in a linear slope

      y = ax + b
    - The intercept **b** of the equation gives the isotopic composition of source 2,

      since for $x \rightarrow 0$ follows $y = b$;
      and also $c = 1/x \cong 1/0 \cong \infty$

      The value b will thus be reached for „infinitely high“ CO_2-concentration.
      Since the contribution of source 1 remains constant (e.g. 360ppm) it is negligible for $c \rightarrow \infty$
      The δ value of source 1 can be deduced from the smallest observed concentrations (i.e. negligible contribution of source 2).

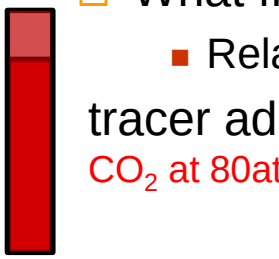


Analysis of tracer experiments

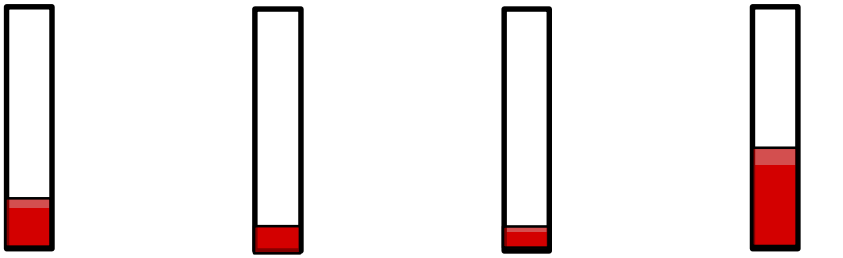
- Two questions can be examined
  - How important is the tracer for the different compartments?
    - What fraction of the compartment is made up of the tracer?
  - How important are the different comparments for the tracer?
    - In which compartments of the system is the tracer allocated?

Analysis of tracer experiments

- Two questions can be examined
  - What fraction of the compartment is made up of the tracer?
    - Relative Specific Allocation (RSA) of the tracer



tracer addition
CO_2 at 80at% ^{13}C

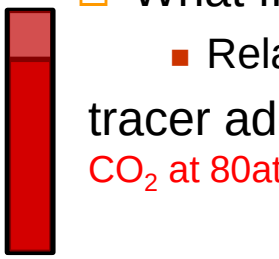


Leaves	Stem	Roots	fine roots
9 at% ^{13}C 10 % RSA	7 at% ^{13}C 8% RSA	6 at% ^{13}C 6 % RSA	30 at% ^{13}C 36% RSA

- The higher the fraction of tracer in a compartment, the higher is growth (or turnover)
- For RSA calculations only tracer enrichment is relevant, but not the amount of tracer applied (or recovered), or compartment size

Analysis of tracer experiments

- Two questions can be examined
  - What fraction of the compartment is made up of the tracer?
    - Relative Specific Allocation (RSA) of the tracer



tracer addition
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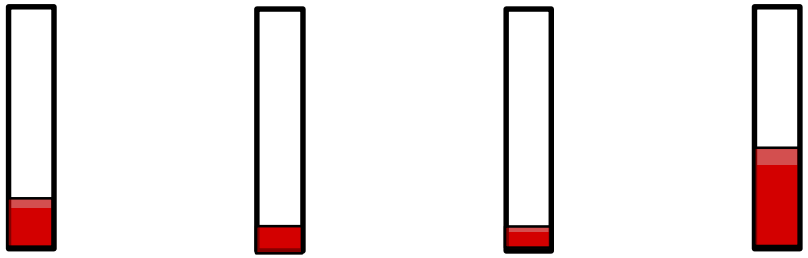


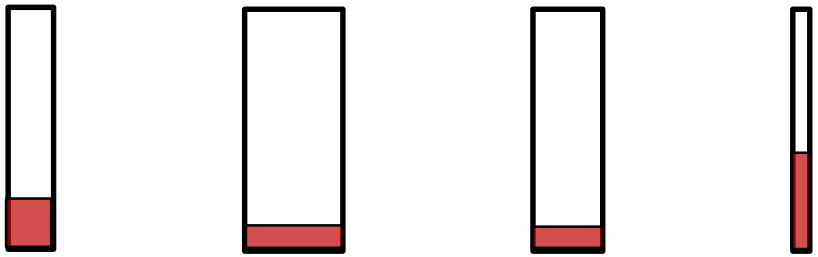
Leaves	Stem	Roots	fine roots
9 at% ^{13}C 10 % RSA	7 at% ^{13}C 8% RSA	6 at% ^{13}C 6 % RSA	30 at% ^{13}C 36% RSA

$$M_L = \left(\frac{at\%_{Mix} - at\%_U}{at\%_L - at\%_U} \right) = \left(\frac{9at\% - 1.1at\%}{80at\% - 1.1at\%} \right) = 10\%$$

Analysis of tracer experiments

- Two questions can be examined
  - In which compartments of the system is the tracer allocated?
    - Tracer partitioning

tracer addition
5 g CO_2 at 80at% ^{13}C



Leaves	Stem	Roots	fine roots
10 % RSA	8% RSA	6 % RSA	36% RSA
5g C	12g C	8g C	2g C

tracer losses
probably as
(root) respiration/exsudation
5g – 2.7g = 2.3 g

Tracer amount = compartment size * Tracer contribution = 5gC * 10% RSA = 0.5g tracer

0.5g tracer	1.0g tracer	0.5g tracer	0.7g tracer	= 2.7g Total tracer recovered

$$\text{Partitioning} = \frac{\text{Tracer amount in compartment}}{\text{Total tracer recovered}} = \frac{0.5\ g}{2.7\ g} = 19\%$$

19%	37 %	19%	26%	Partitioning

- A measure for the relative sink strength of the compartments

Analysis of tracer experiments

- Two questions can be examined
  - What fraction of the compartment is made up of the tracer?
    - Relative contribution of the tracer to the compartment
      - Relative specific allocation (RSA)

$$RSA = \frac{(at\%_{Mix} - at\%_{U})}{(at\%_{L} - at\%_{U})}$$

  - In which compartments of the system is the tracer allocated?
    - Partitioning of the tracer

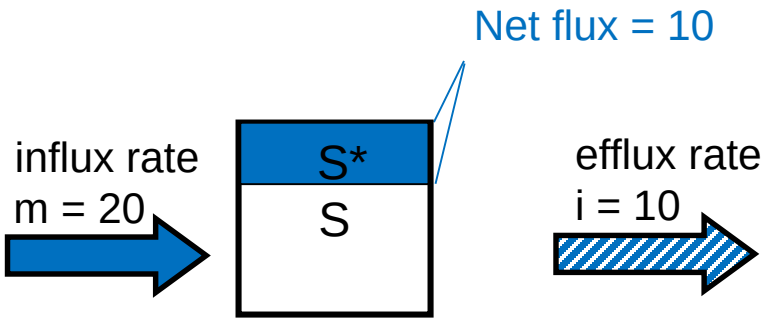
$$Partitioning = \frac{\text{Tracer amount in compartment}}{\text{Total tracer recovered}}$$

- Absolute amount of tracer in the compartment

Tracer amount = compartment size * RSA

Labelling techniques

- Continuous labelling can only elucidate net fluxes (turnover rates):
  Tracer will be incorporated into the pool of interest, but may also leave the pool



Labelling techniques

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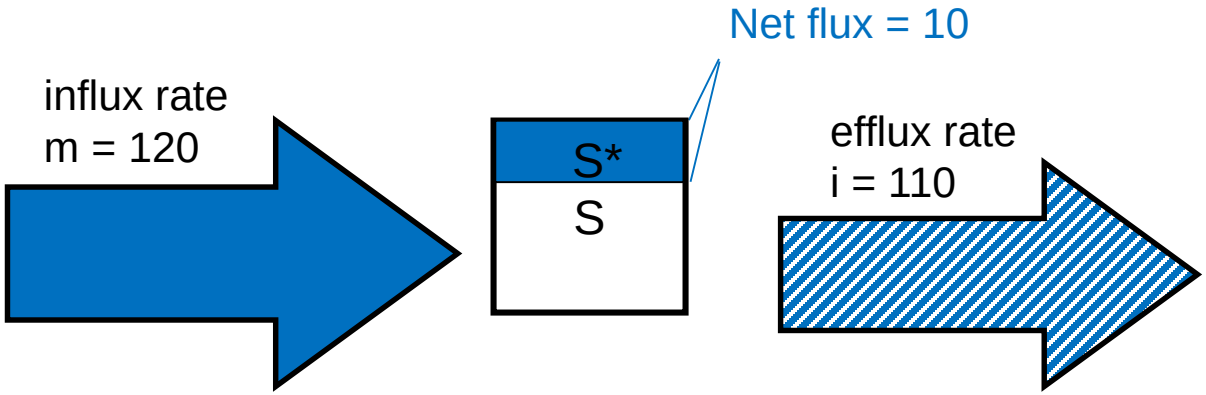
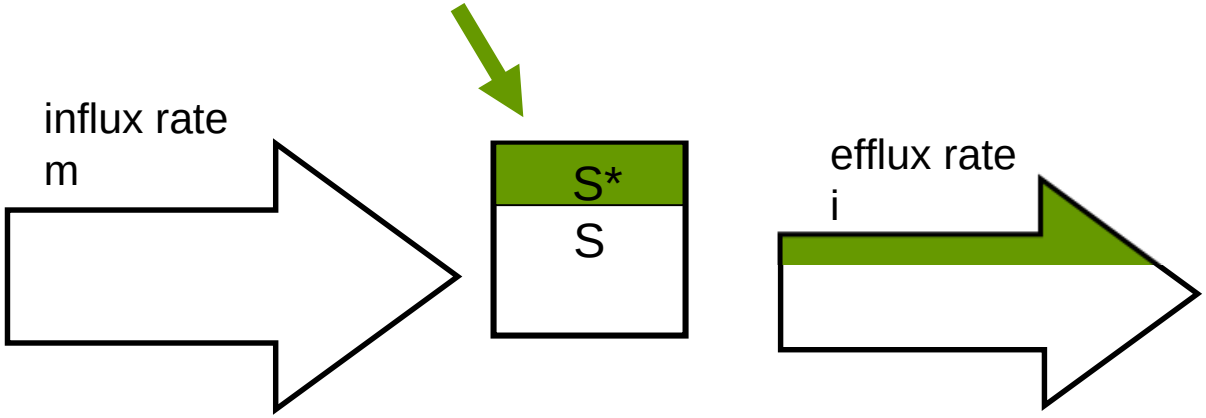
  - Tracer incorporation will indicate the sink strength of the pool but not its turnover



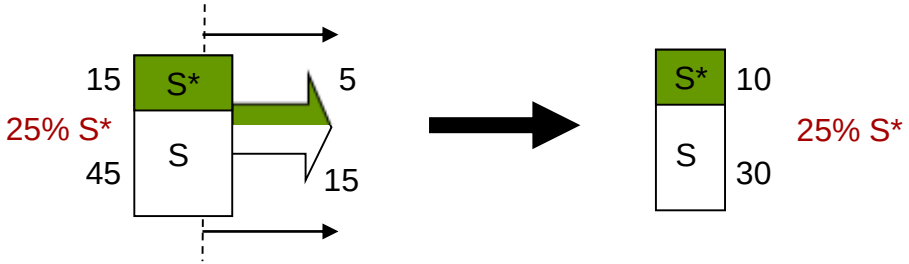
Labelling techniques – isotope pool dilution

- Continuous labelling can only elucidate **net fluxes** (turnover rates):
  Tracer will be incorporated into the pool of interest, but may also leave the pool
  - Tracer incorporation will indicate the sink strength of the pool but not its turnover
- To determine **gross flux rates** (influx rate m, efflux rate i) the pool S is homogeniously isotopically labelled
  - As a consequence the efflux i from the pool is isotopically labelled
    but the influx m is not.
- This approach ist called **Isotope Pool Dilution**

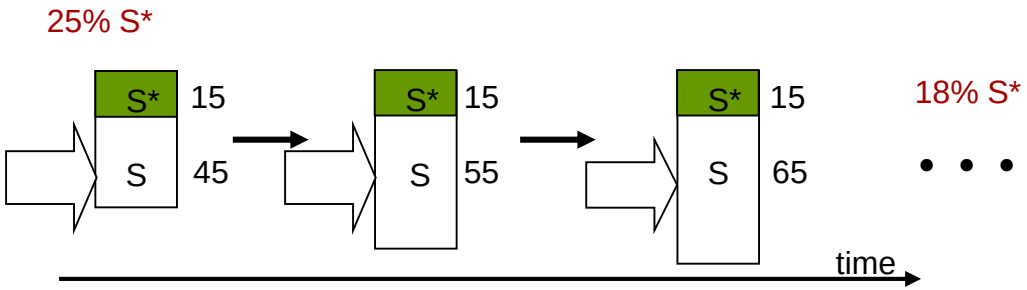


Labelling techniques – isotope pool dilution

- Efflux from the pool (rate i) does not change isotopic composition of the pool, since both labelled and unlabelled substance is lost



- Influx of new unlabelled substance (rate m) will change (dilute) isotopic composition of the pool



Labelling techniques

- Isotope pool dilution
  - **Prerequisites**
    - The examined pool is labelled homogeniously
    - All processes obey a zero order kinetic (i.e. rates are constant and independent of pool sizes)
    - Efflux and influx are fractionation free (or labelling is high enough to minimize this effect)
    - Labelled substance that leaves the pool will not enter the pool again
  - Influx rate **m** and efflux rate **i** can be calculated according to

$$m = \frac{S_0 - S}{t} \frac{\ln \frac{S_0^* S}{S^* S_0}}{\ln \frac{S_0}{S}}, \quad i \neq m \qquad i = \frac{S_0 - S}{t} \frac{\ln \frac{S_0^*}{S^*}}{\ln \frac{S_0}{S}}, \quad i \neq m$$

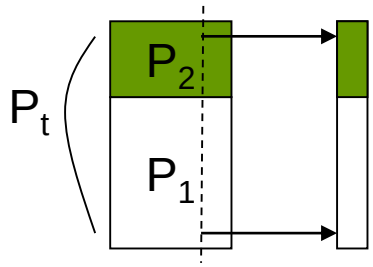
$$m = i = \frac{S_0}{t} \ln \frac{S_0^*}{S^*} \quad i = m$$

with S = substrate, S^* = labelled substrate,
subscripts t and 0 refer to the points in time t and t=0

(after Kirkham & Bartholomew 1954)

Labelling techniques

- To determine the size of a pool, a known amount of labelled substance is added to that pool. The amount of enrichment in the total pool indicates the size of the total pool without the need to extract the complete pool.



$$P_t \cdot at\%_t = P_1 \cdot at\%_1 + P_2 \cdot at\%_2$$

and also

$$P_t = P_1 + P_2$$

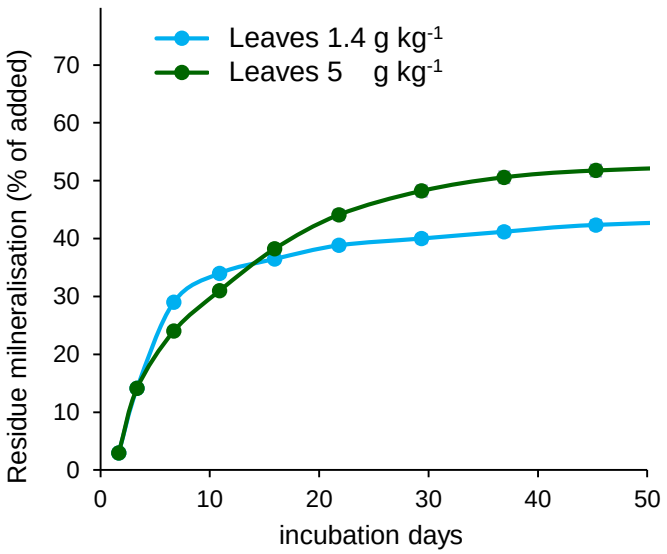
$$\Rightarrow P_1 = \frac{P_2 \cdot at\%_2 - P_2 \cdot at\%_t}{(at\%_t - at\%_1)}$$

P: pool size

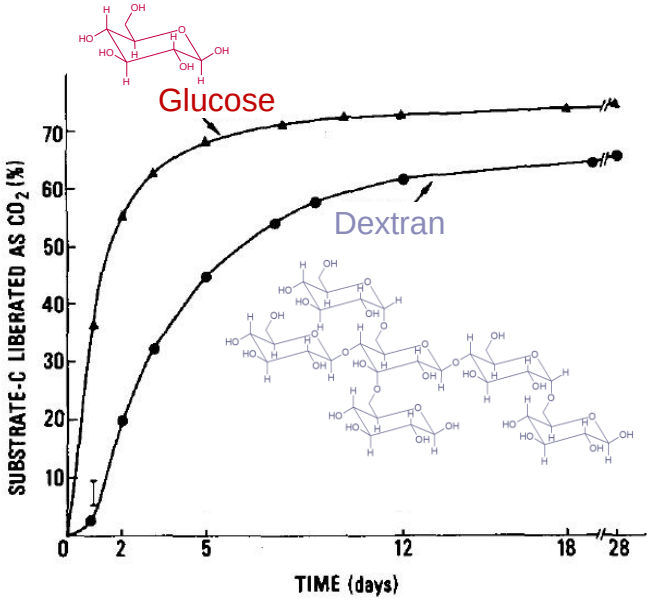
at%: enrichment of the pool

Labelling techniques

- What requirements must be met?
  - Will the system be disturbed by adding the labelling substance?
    - E.g. substrate addition amount alters substrate partitioning in soil
  - Is the added tracer a good model substrate?
    - E.g. substrate partitioning depends on substrate quality



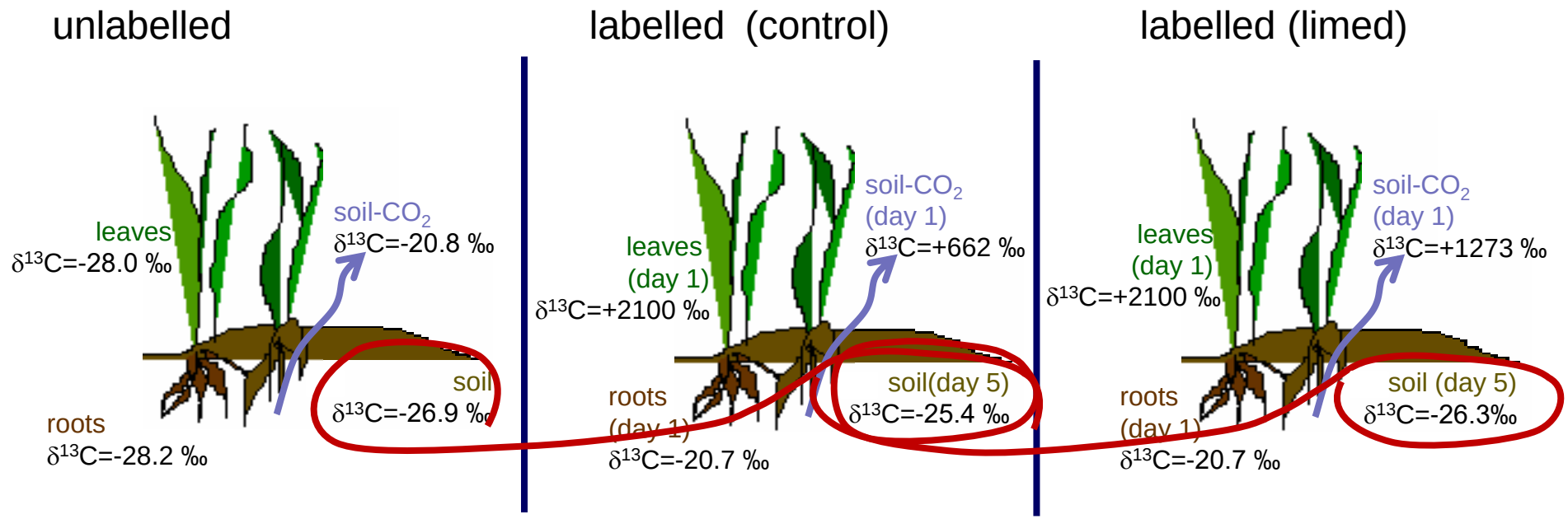
Shahbaz et al. 2016



Oades & Wagner 1971

Labelling techniques

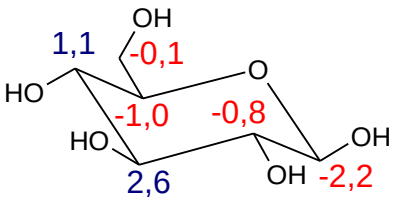
- What requirements must be met?
  - Will the system be disturbed by adding the labelling substance?
  - Is the labelling high enough in the target compartment?



Staddon 2004 Trends Ecol Evol

Labelling techniques

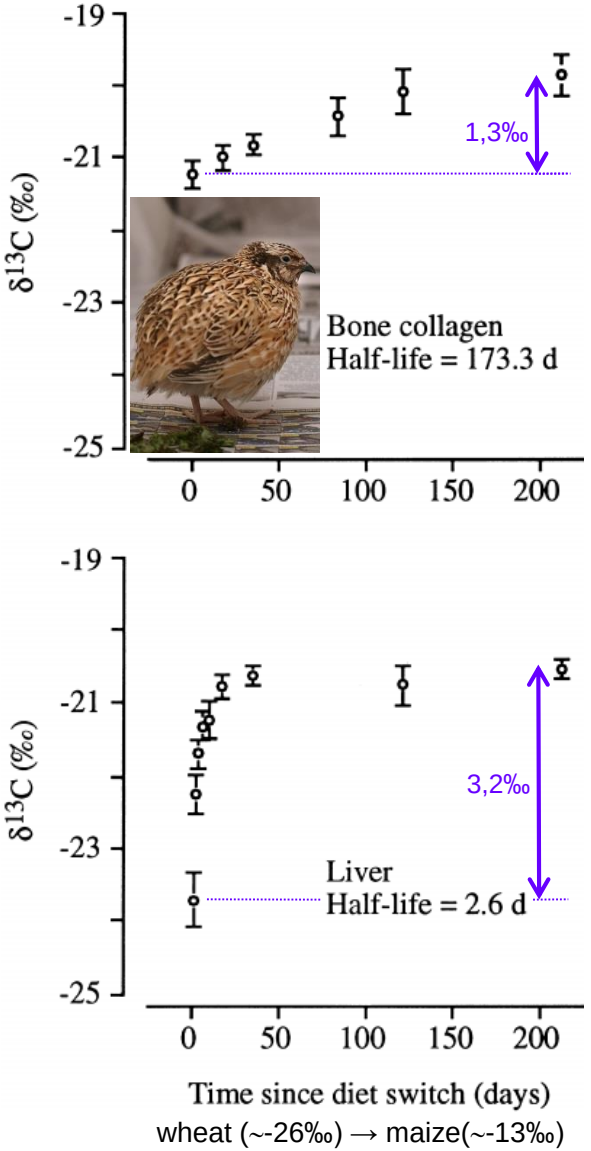
- Which requirements must be met?
  - Is the labelling in the target compartment high enough?
  - Will the system be disturbed by adding the labelling substance?
- Possible systematic errors
  - Fractionation
    - Differences between pools will be over- or underestimated if the transition between pools is subject to fractionation and is not corrected for
    - Fractionation during sample preparation can lead to erroneous results (e.g. precipitation of CO_2 as carbonate, derivatisation reactions for compound specific analysis, …)
      - When working with high enrichments, fractionation effects can be neglected
  - Molecules are labelled non-uniformly (e.g. site specific isotopic composition in sugar)



Site specific differences in delta value between C_3 and C_4 glucose (deviation from mean)

Labelling techniques

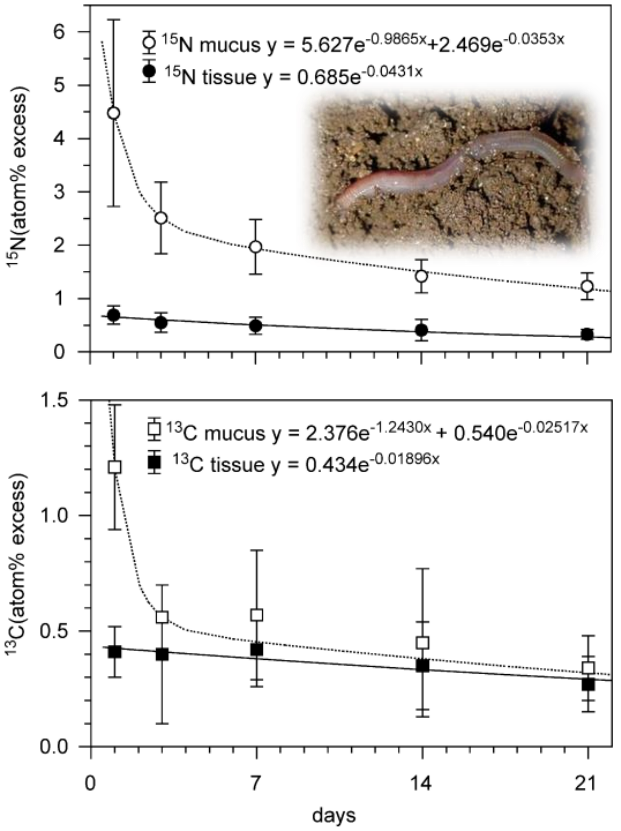
- Possible systematic errors
  - Non-uniform labelling
    - Different compartments of plants or animal have different turnover times, i.e. the label is taken up at different rates
      - „Fast“ pools are labelled more strongly than „slow“ pools
      - „Very slow“ pools cannot be observed in „too short“ experiments
        → Δ(wheat/maize) = ~ 13‰
        → Δ(liver$_{t=200d}$) = 3,2‰



Gannes, del Rio & Koch 1998 Comp. Biochem. Physiol.

Labelling techniques

- Possible systematic errors
  - Non-uniform labelling
    - Different compartments of plants or animal have different turnover times, i.e. the label is taken up at different rates
      - „Fast“ pools are labelled more strongly than „slow“ pools
      - In decompostition studies, „fast“ pools are overrepresented
        - e.g. strong decline of label in mucus, but mucus makes up only very small part of earthworm biomass
      - „Slow“ pools cannot be observed

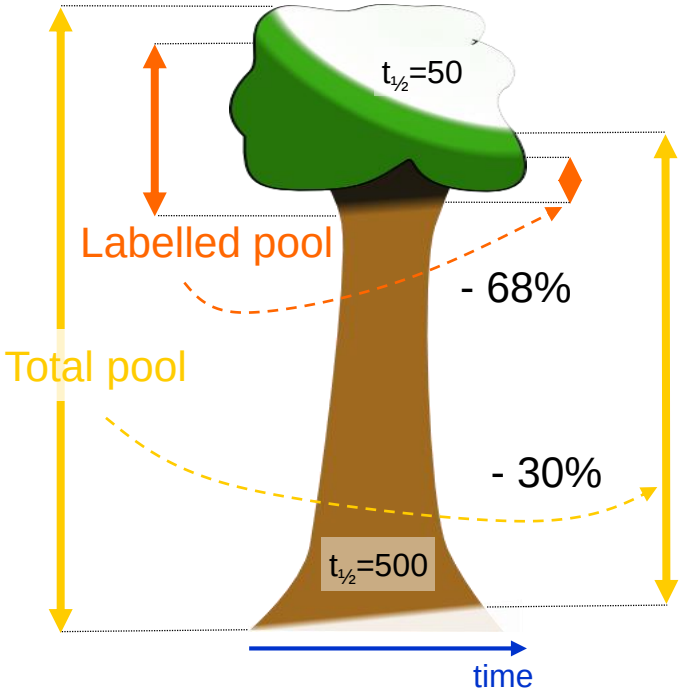


→ **Determination of pool numbers and sizes will be erroneous if non-uniformly labelled organisms are observed**

Dyckmans et al. 2004 Soil Biol Biochem

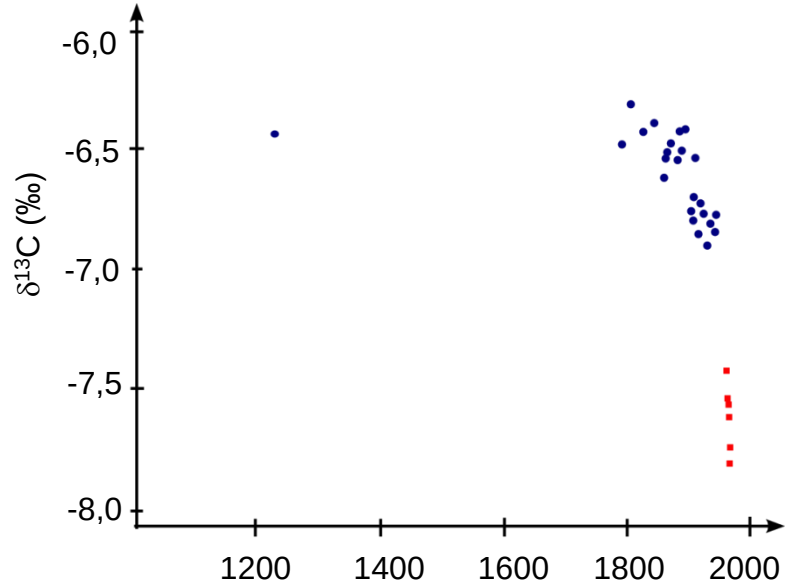
Labelling techniques

- Possible systematic errors
  - Non-uniform labelling
    - Labelled plants (or animals) that are to be employed as a tracer may be differently labelled in different compartments (e.g. free sugars are more strongly labelled than cellulose, leaves more than wood)
      - „Fast“ pools are more enriched than “slow” pools when labelling is not complete.
        If these pools are decomposed disproportionately fast, isotope analysis will overestimate total plant turnover

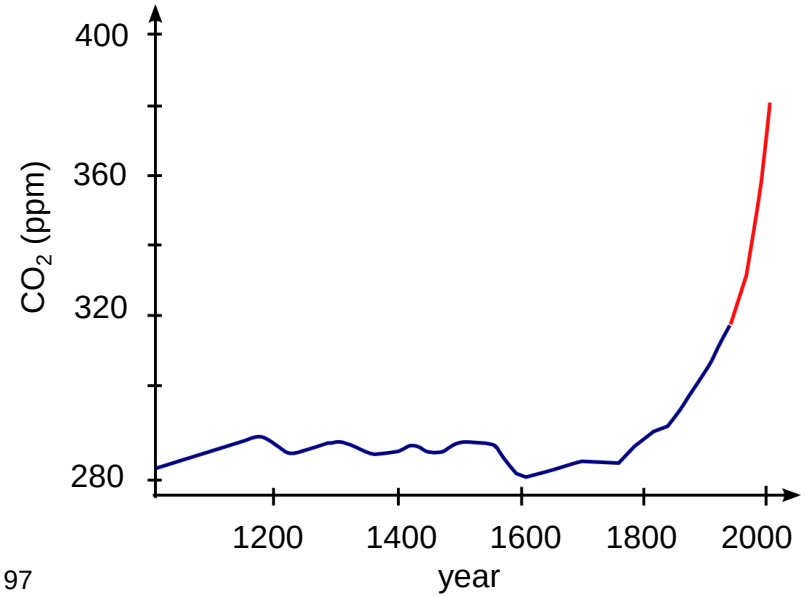


→ **The determination of turnover rates will be erroneous if non-uniformly labelled tracers are employed**

Historic CO_2-data



$\delta\,^{13}C$ (‰) in atmospheric CO_2



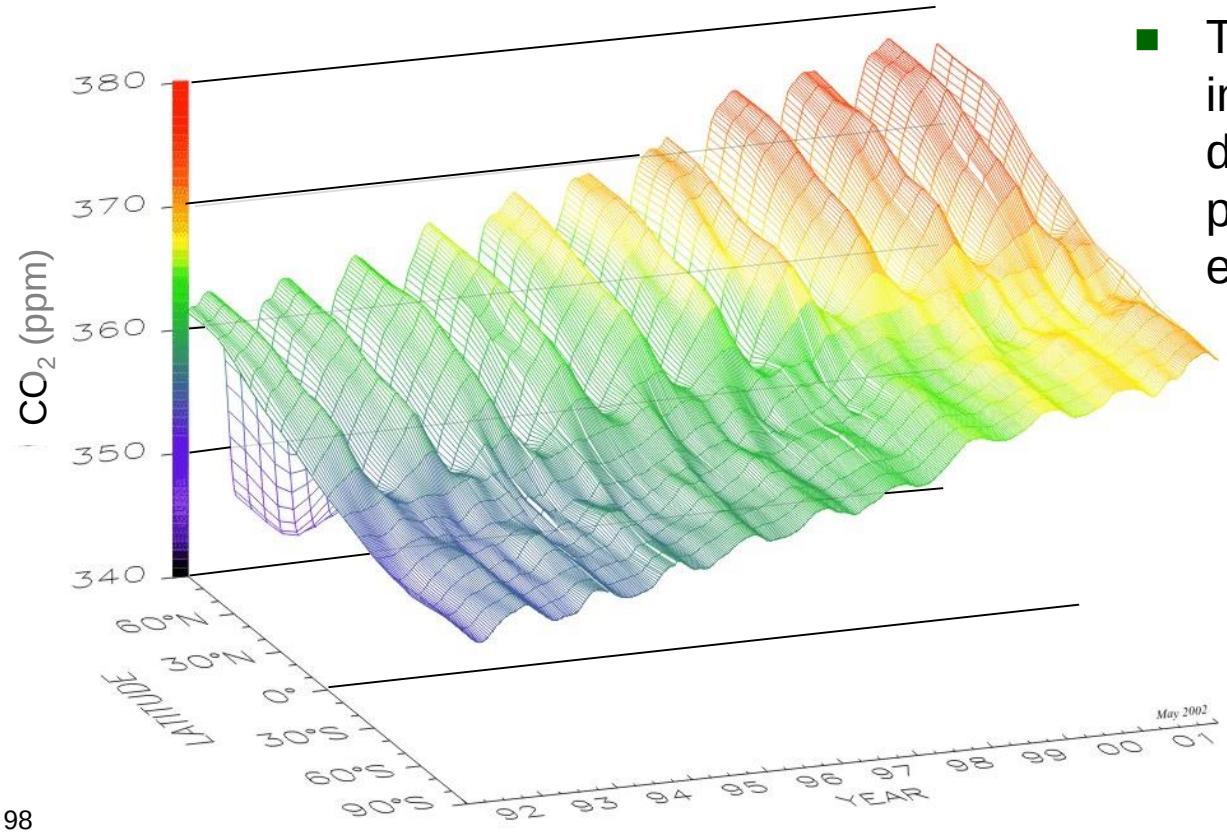
atmospheric CO_2-concentration

- determined in ice cores
- determined in the atmosphere in Hawaii (Mauna Loa)

Siegenthaler and Oeschger, 1987

CO_2-concentration in the atmosphere

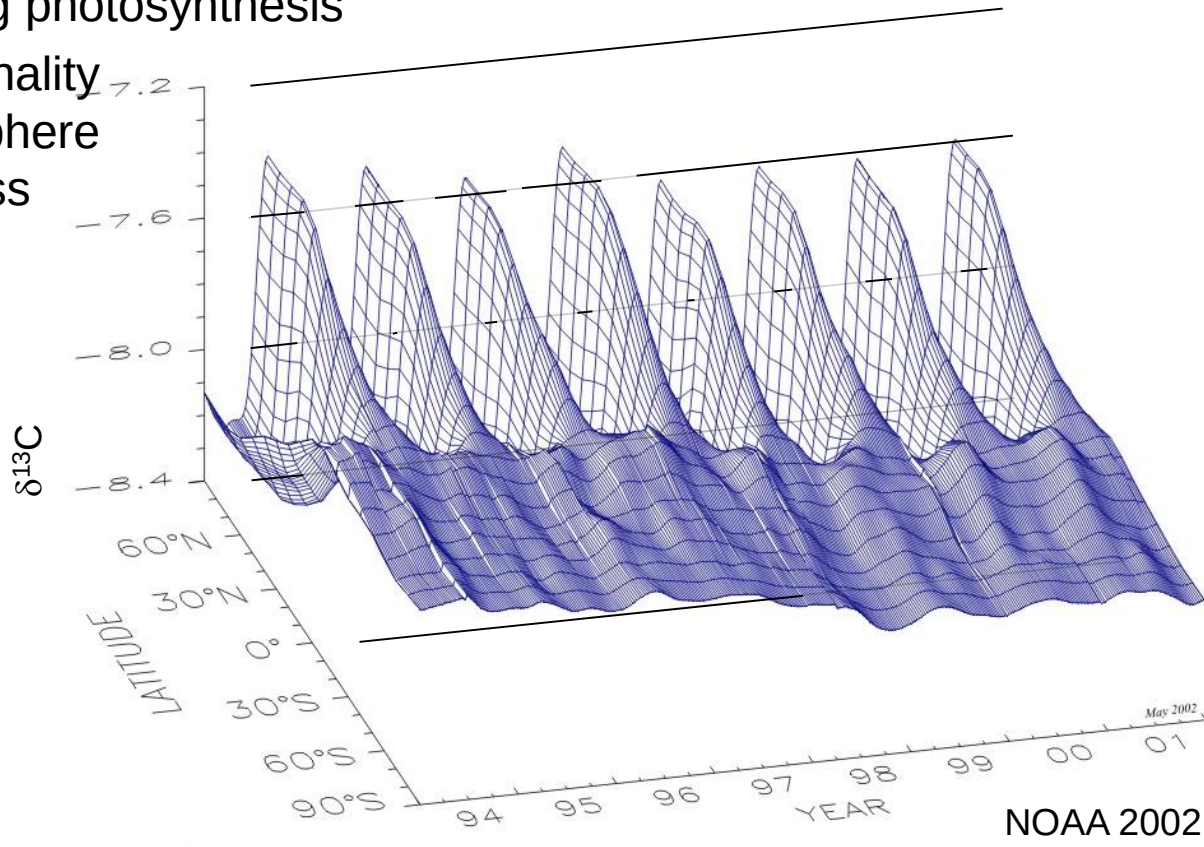
- The mean atmospheric CO_2-concentration rises continuously as a result of fossil fuel burning
- The atmospheric CO_2-concentration shows a distinct seasonality, it decreases during the summer due to plant photosynthesis (and lower emissions)



- The seasonalty is less distinct in the southern hemisphere due to lower landmass (less photosynthesis) and higher exchange with the ocean

Seasonality of $\delta^{13}CO_2$ in the atmosphere

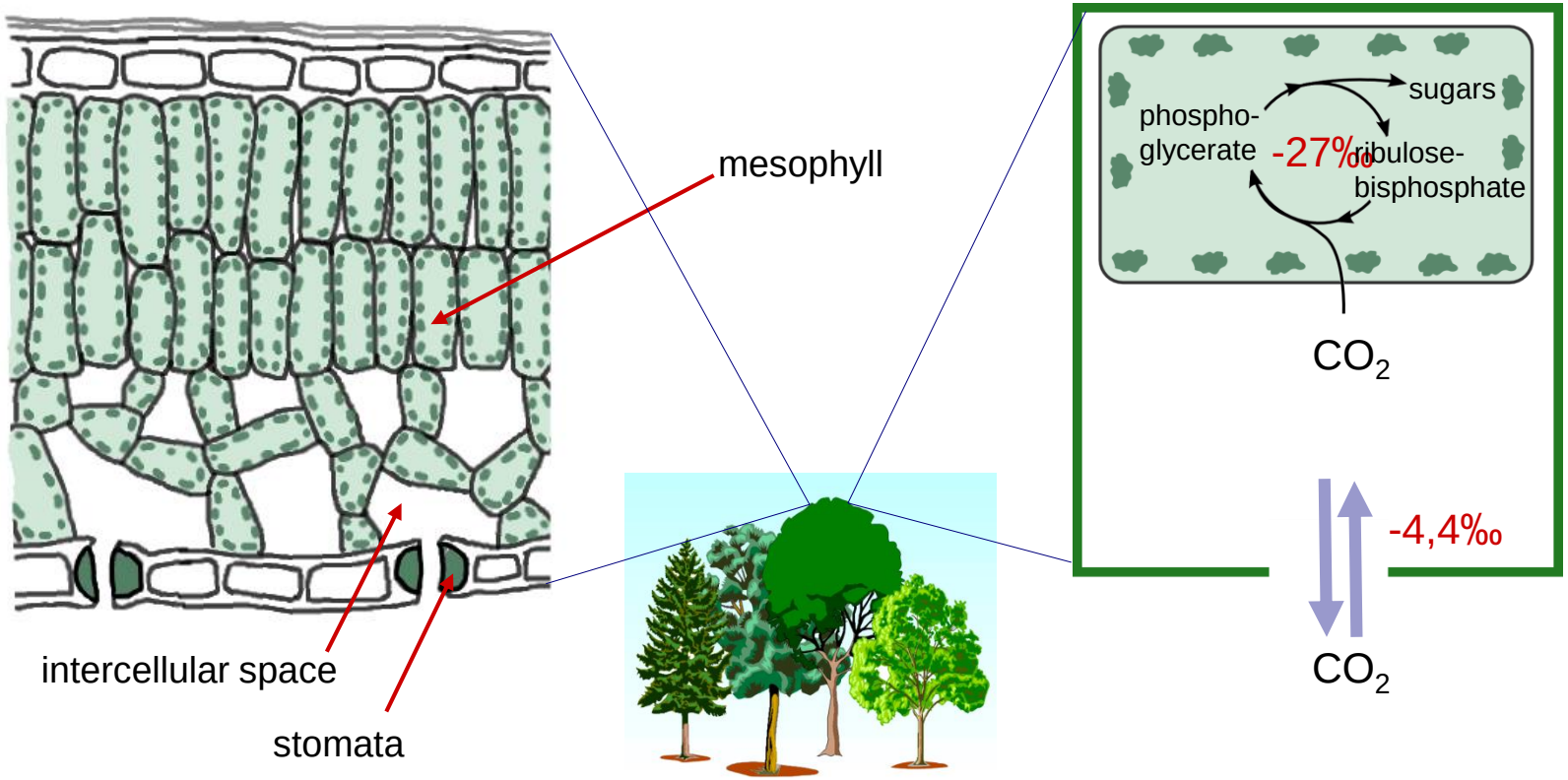
- The $\delta^{13}C$ of atmospheric CO_2 becomes continouosly more negative as a result of the lower $\delta^{13}C$ of fossil fuels
- The $\delta^{13}C$ of atmospheric CO_2 shows a distinct seasonality in the northern hemisphere
  - Fractionation during photosynthesis
- The less distinct seasonality on the southern hemisphere is due to lower landmass (less photosynthesis) and higher exchange with the ocean



NOAA 2002

^{13}C-fractionation - C_3-photosynthesis

- Photosynthesis of C_3 plants



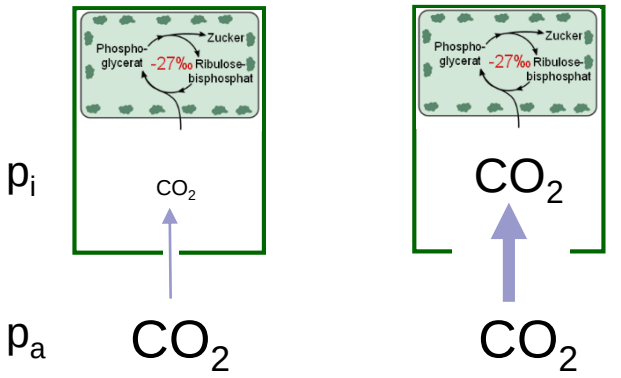
- Fractionation: a) CO_2-diffusion through the stomata (-4,4 ‰)
  b) RuBisCO (-27 ‰)
  ⇒ Net effect (~ -13 … -22 ‰)

Fractionation model

- ^{13}C-fractionation of C_3 plants

$$\Delta = a + (b - a)\frac{p_i}{p_a}$$

Δ = discrimination
a = fractionation by diffusion (-4,4 ‰)
b = fractionation by RuBisCO (-27 ‰)
p_i = CO_2-concentration in the intercellular space
p_a = CO_2-concentration in the atmosphere



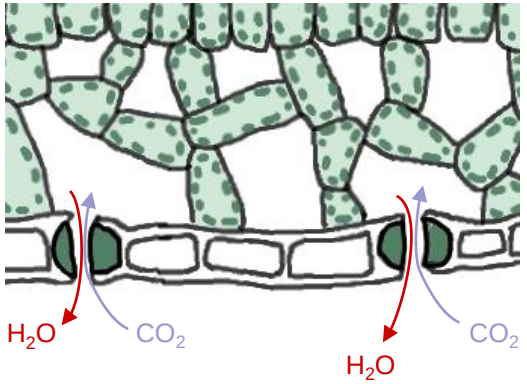
p_i/p_a small ⇒ Δ small

p_i/p_a big ⇒ Δ big

- p_i/p_a is controlled primarily by stomatal conductance
- Smaller p_i/p_a leads to smaller isotope discrimination Δ
- With increasing p_i/p_a fractionation by RuBisCo becomes more important

Farquhar 1983

Water use efficiency“ (WUE) and $\delta^{13}C$



- Water use efficiency (WUE) is the relation between CO_2-uptake and water loss through the stoma

- WUE = mmol CO_2 fixed / mol H_2O transpired

  WUE ~ 0,8 – 1,5 mmol CO_2 / mol H_2O (for C_3-plants)

  - Water loss increases proportionally with stoma aperture, as the water vapor gradient between the intercellular space and the atmosphere is not affected by stoma aperture
  - The photosynthetic rate decreases sub-proportionally when stomata are closed since RubisCO is very effective at low CO_2-concentrations

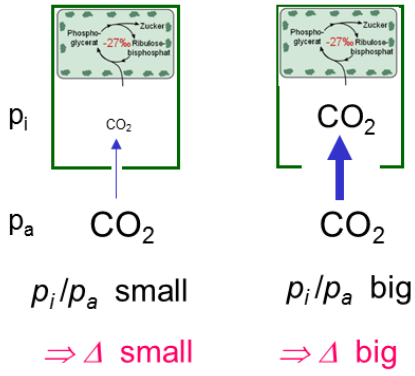
→ WUE increases with decreasing stomatal conductance, i.e. with closing stomata

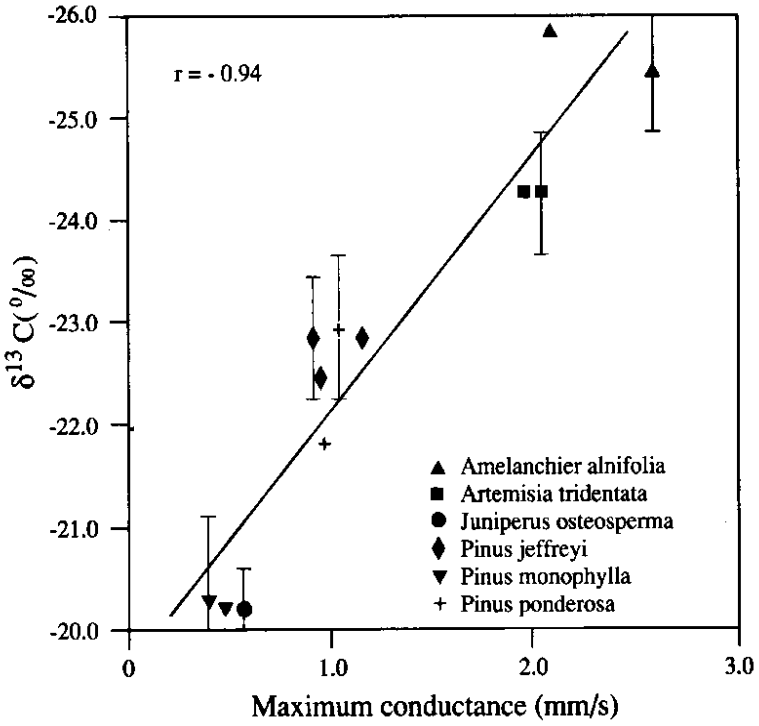
Water use efficiency“ (WUE) and $\delta^{13}C$

- In C_3-plants the δ ^{13}C-value will decrease with increasing stomatal conductance.
  (given constant CO_2-concentration and light)

- This relationship between fractionation and stomatal conductance in C_3-plants results in a relationship between $\delta^{13}C$ and WUE

  → the δ ^{13}C-value of C_3-plants can serve as an index for WUE

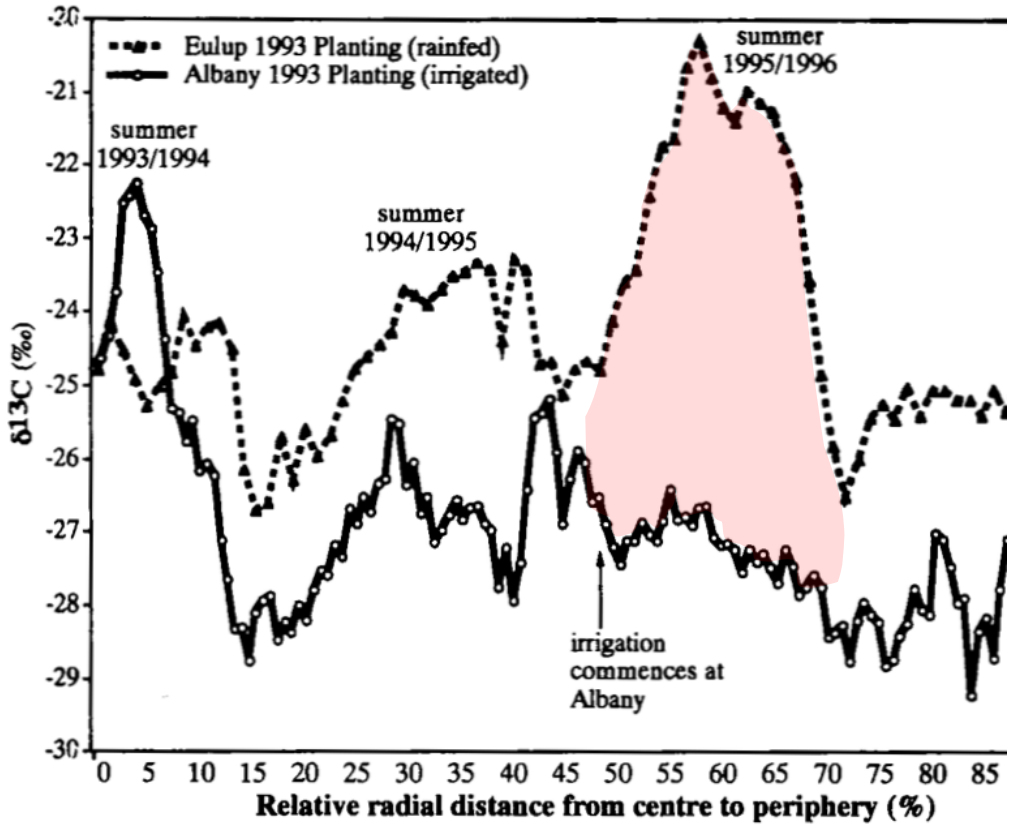




Delucia et al. 1988

Water availability and $\delta^{13}C$ in stem wood

- δ ^{13}C-values in stem wood of *Eucalyptus globulus*



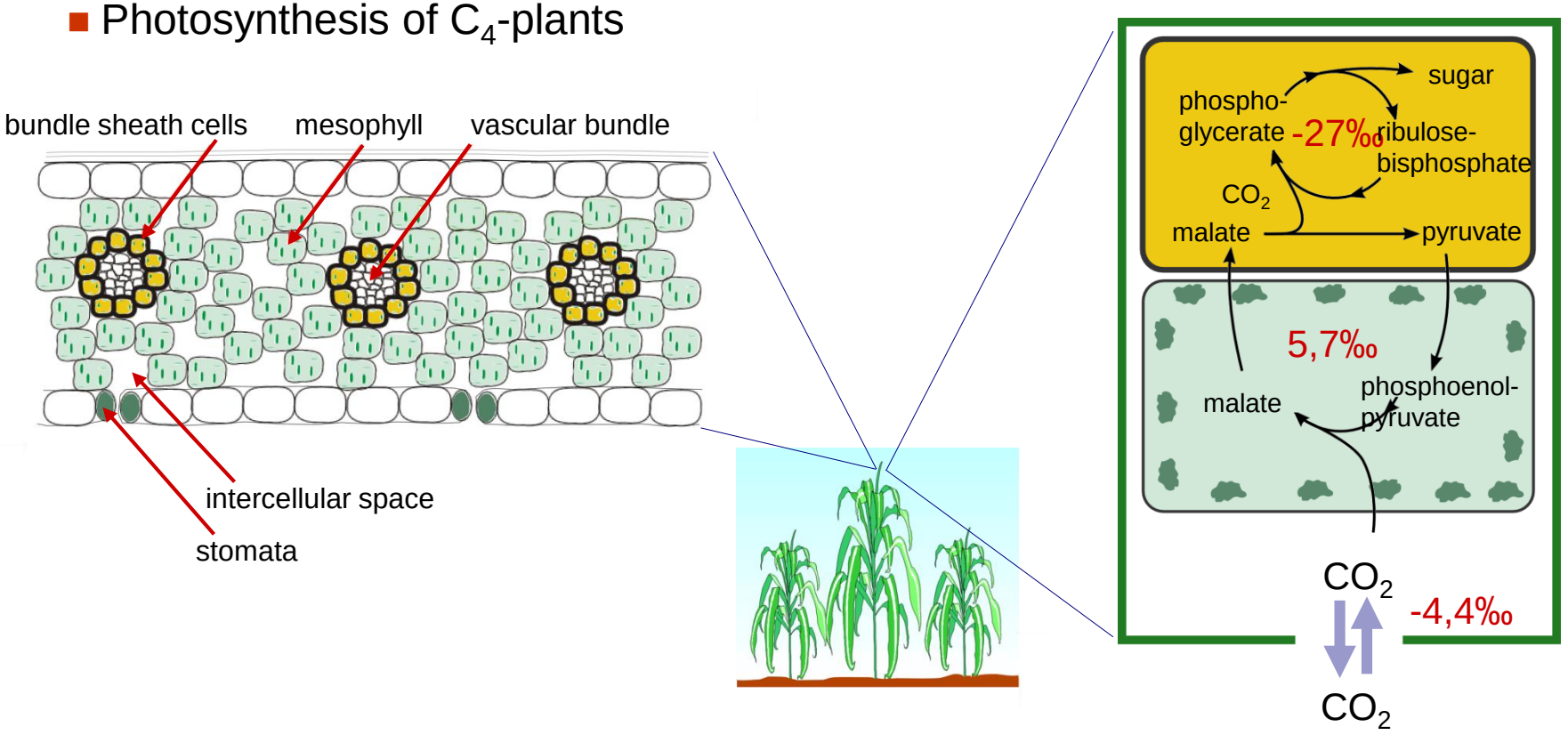
→ Irrigation increases stomatal conductrance and ^{13}C-discrimination

→ The increase in $\delta^{13}C$ during summer is suppressed by irrigation.

Pate and Arthur 1998

^{13}C-discrimination - C_4-photosynthesis

- Photosynthesis of C_4-plants



- Fractionation: a) CO_2-diffusion through the stomata (-4,4 ‰)
  b) PEP-carboxylase (5,7 ‰)
  c) RuBisCO (-27 ‰)
  ⇒ Net effect (~ -1 … -9 ‰)

Fractionation model

- ^{13}C-discrimination in C_4-plants

$$\Delta = a + (b_4 + b_3\,\phi - a)\,\frac{p_i}{p_a}$$

Δ = discrimination

a = Fractionation by diffusion (-4,4 ‰)

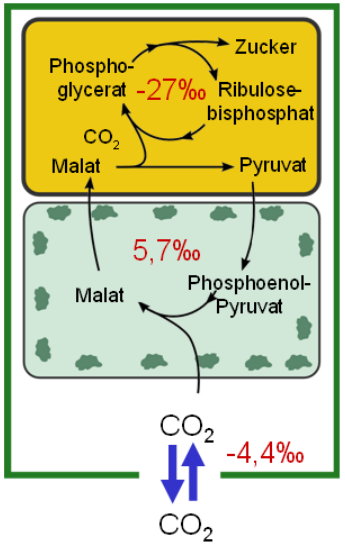
b_4 = Fractionation by PEP-carboxylase (5,7 ‰)

b_3 = Fractionation by RuBisCO (-27 ‰)

ϕ = Fraction of PEP-fixated C, that leaks as CO_2 (usually ranges about 0,2 to 0,3)

p_i = CO_2-concentration in the intercellular space

p_a = CO_2-concentration in the atmosphere



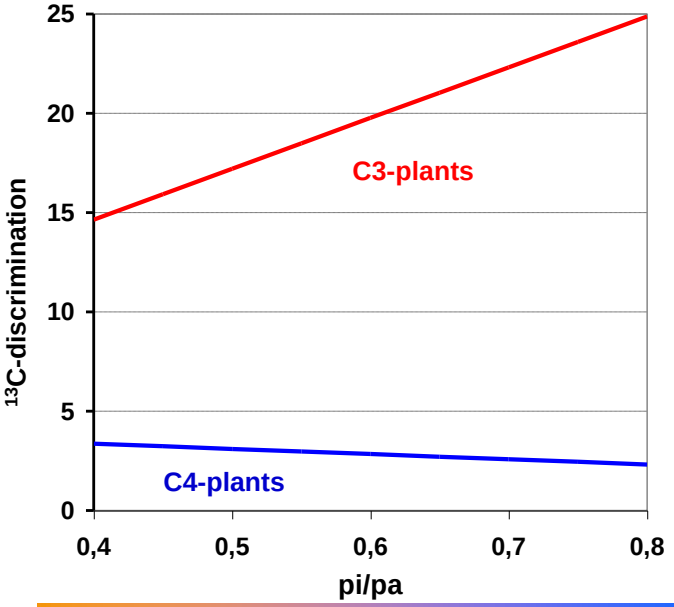
- With increasing p_i/p_a isotope disrimination Δ decreases
  - The effect of p_i/p_a is opposite to that for C_3-plants
  - The effect ist much smaller than for C_3-plants because PEP and RuBisCO discrimination works in opposite directions

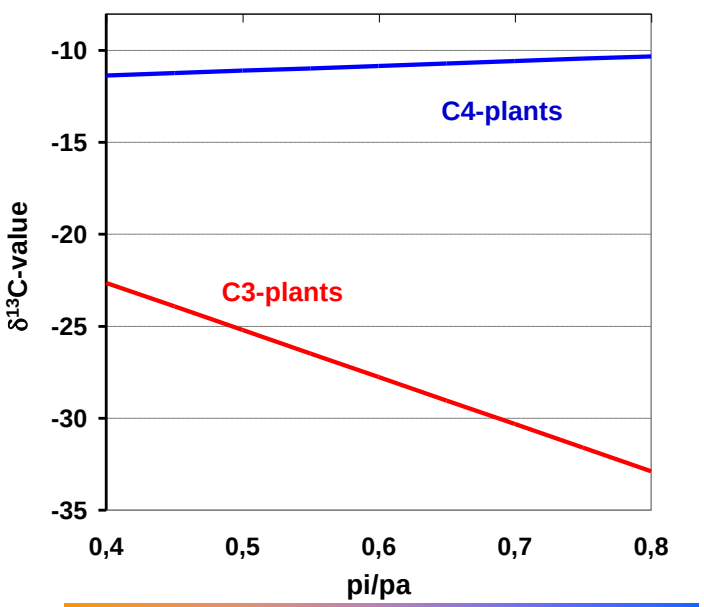
Farquhar 1983

Driving forces for the $\delta^{13}C$-value

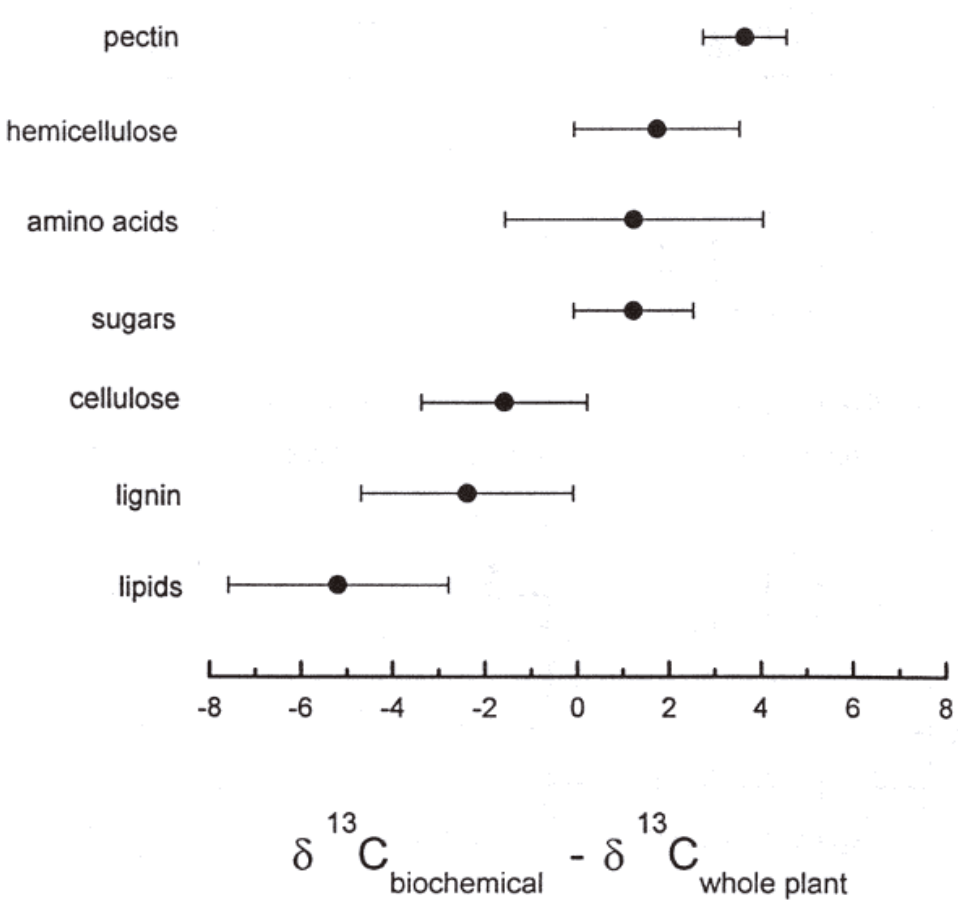
- Variables influencing p_i/p_a:

Environmental variables	Biological variables
irradiance, CO_2-conc.	genetic variations
water vapor deficit	habit
water availability, soil type	competition
salt concentration in the soil	developmental stage
altitude, exposition	photosynthetic capacity





$\delta^{13}C$ of biochemical fractions in plants

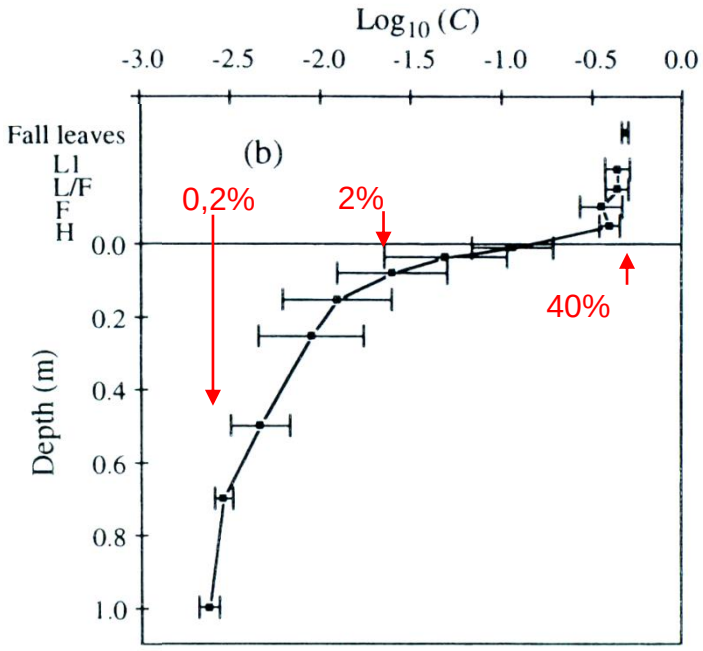


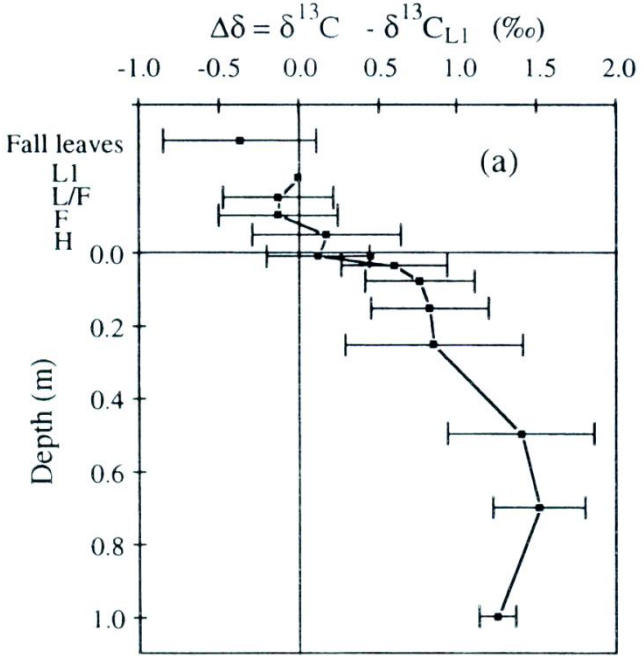
- Biochemical fractions influence the $\delta^{13}C$-value of the plants
- Pectin, amino acids and hemicelluloses are enriched in ^{13}C
- Lignin and lipids are depleted in ^{13}C
- The $\delta^{13}C$-value of plant compartments will change if the amount of an isotopically distinct biochemical fraction is altered

Deines, 1980

$\delta^{13}C$ of soil organic matter

- Carbon concentration decreases strongly with depth due to decomposition (microbial respiration)
- $\delta^{13}C$ of C_{org} increases with increasing soil depth
  - Fractionation during microbial C_{org}-respiration
  - Selective decomposition of isotopically distinct substances
  - „Old C“ differs isotopically from „new C“ (altered $\delta^{13}C_{atm}$)





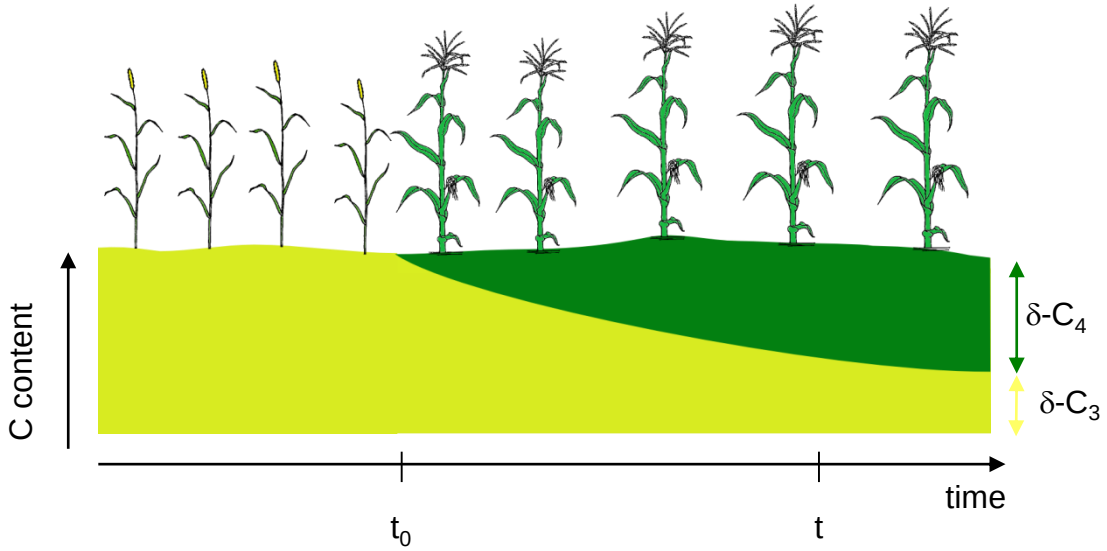
C-dynamic in soil – methods

- Labelling of „new“ C-input
  - Change from C_3- to C_4vegetation
    - e.g. wheat → maize


  - Free Air Carbon Enrichment
    - Artificial increase of air CO_2 concentration to study impact of global change on plants

C-dynamic in soil – $C_3 \rightarrow C_4$ change

- Change from C_3- to C_4-vegetation
  - If C_3-vegetation on a soil is replaced by C_4-plants, the contribution to soil C of the plants are distinguishable due to their isotopic composition.
  - „Old“ C derived from C_3-plants in the soil will gradually be replace by „new“ C derived from C_4-plants.
  - The isotopic composition of a pool shows how much of the „old“ (C_3-derived) C has been replaced (two pool mixing model). From that value the turnover time of the carbon can be derived.



$$M_{C_4} = \left(\frac{\delta_{Mix} - \delta_{C_3}}{\delta_{C_4} - \delta_{C_3}} \right)$$

Kompetenzzentrum Stabile Isotope
Universität Göttingen

Free Air Carbon Enrichment (FACE)

Ambient Air
CO_2 = 380 ppm
$\delta^{13}C$ = -8.5‰

mixing

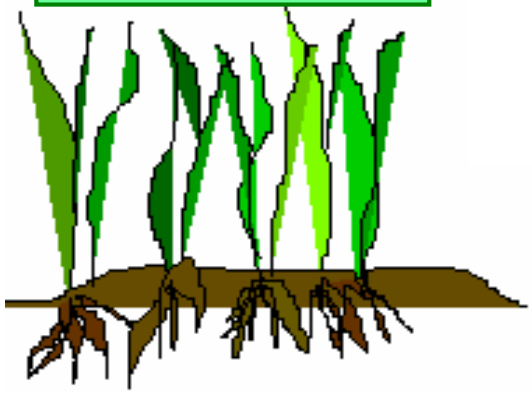
FACE Air
CO_2 = 760 ppm
$\delta^{13}C$ = -24‰

mixing

Tank Air
CO_2 = 100%
$\delta^{13}C$ = -40‰

Photosynthesis
Δ =-19‰

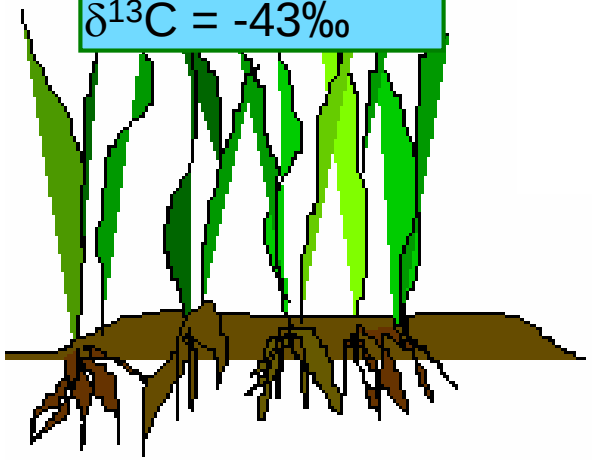
Ambient C_3 Plant
$\delta^{13}C$ = -28‰



Ambient Soil
$\delta^{13}C$ = -27‰

Photosynthesis
Δ =-19‰

FACE C_3 Plant
$\delta^{13}C$ = -43‰

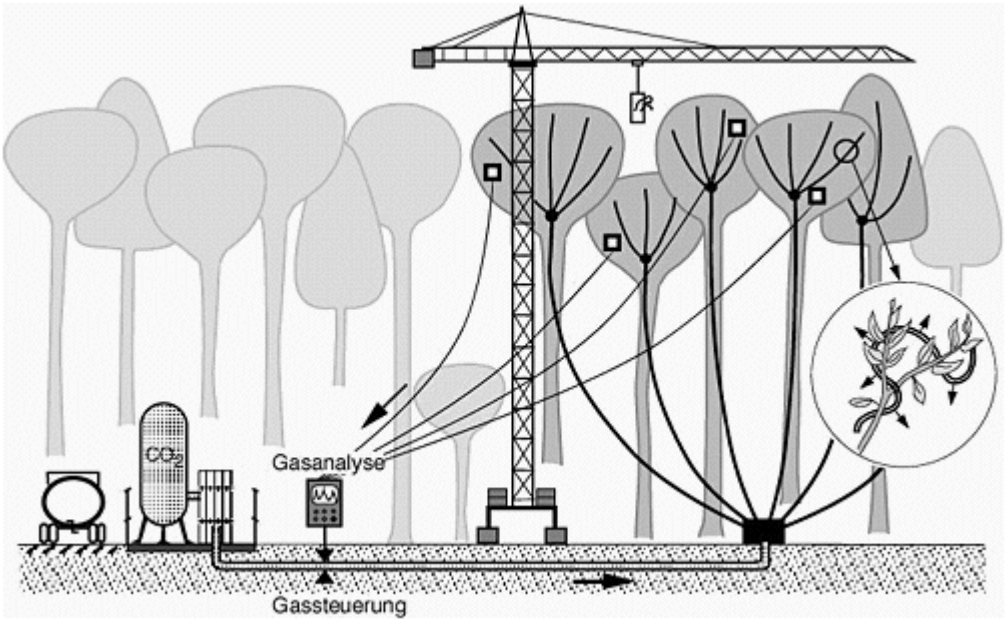


FACE Soil
$\delta^{13}C$ = -29‰

- Introduction of additional CO_2 with different isotopy
  - Studying the plants reaction to increased atmospheric CO_2 concentration.
    Studying the flux of „new“ C within the plant-soil system

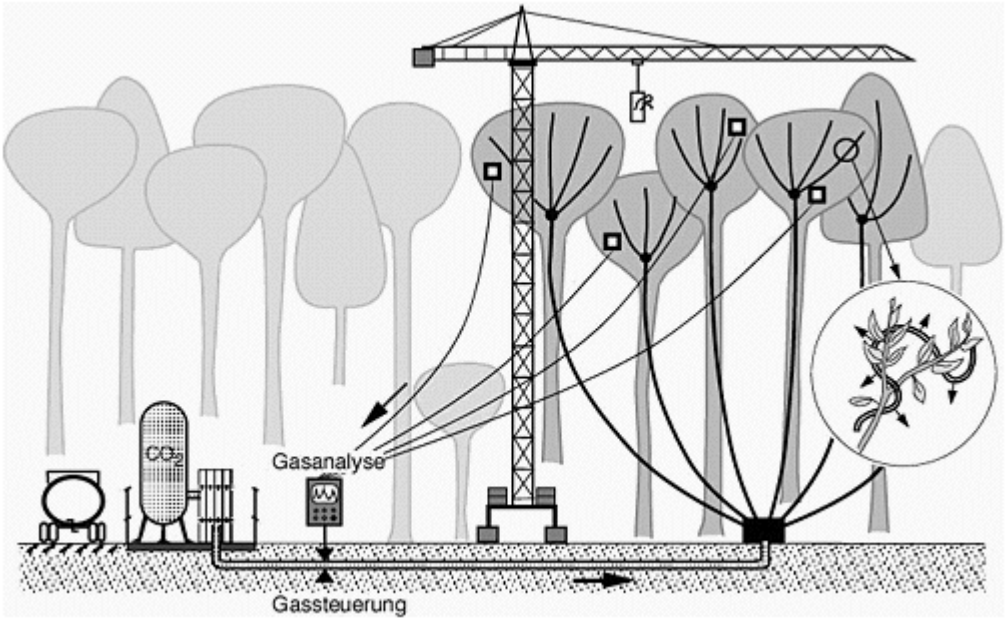
FACE – Swiss Canopy Crane Project

- Beech wood near Basel
  - Fumigation of a 100 years old beech stand with CO_2 through a pipe system
  - Added CO_2 is depleted in ^{13}C



FACE – Swiss Canopy Crane Project

- Beech wood near Basel
  - Fumigation of a 100 years old beech stand with CO_2 through a pipe system
  - Added CO_2 is depleted in ^{13}C
    - Monitoring of actual CO_2-Isotopy in the canopy with C_4-plants as „isometer“ (^{13}C fractionation of C_4 plants is fairly constant independently of stoma aperture)



Problems with FACE

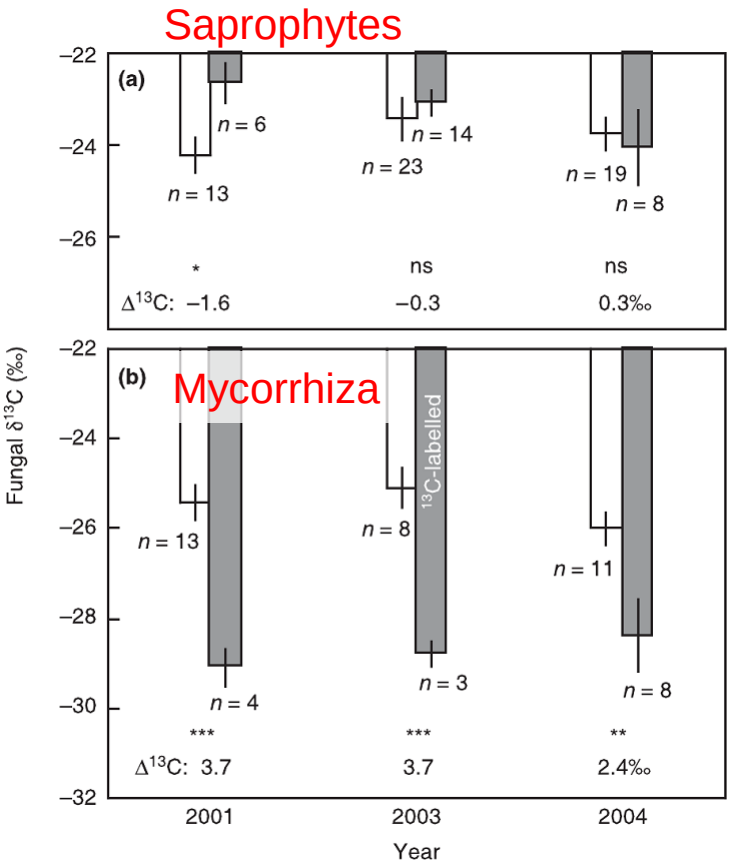
- Change of isotopic composition in the air (and thus in the plant compartment) is relatively small (approx. 20‰)
- C-dynamic of most soil pools is rather slow

  $\Rightarrow$ Isotopic differences in soil pools are imprecise or not significant

e.g. SCC: No significant C labelling in the decomposing fungi (saprophytes) in the soil,

but in the mycorrhiza of plant roots

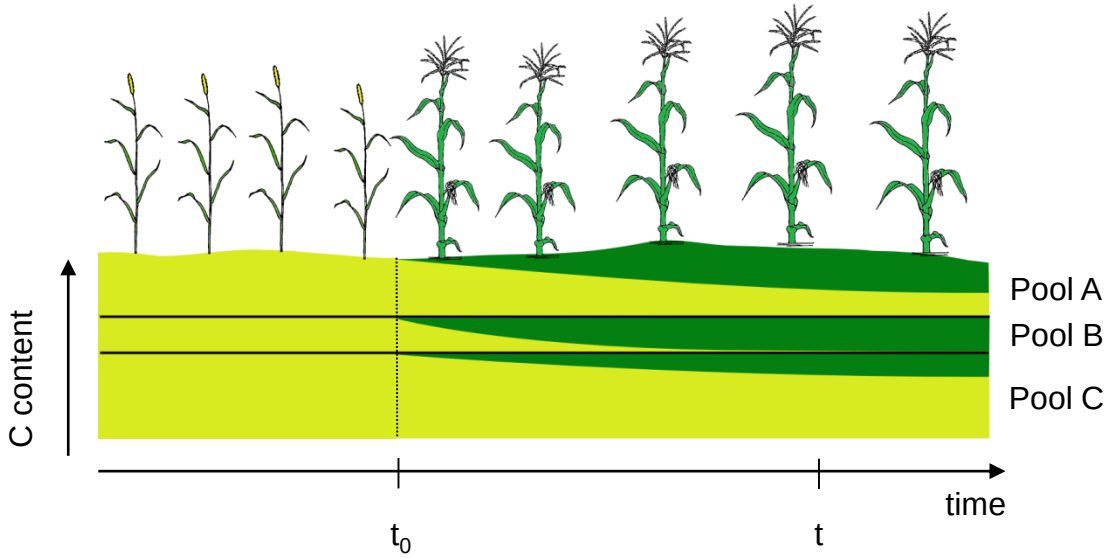
- C-dynamic can only be traced accurately after long periods of label input
  - $C_3 \rightarrow C_4$ change experiments are >40 years old

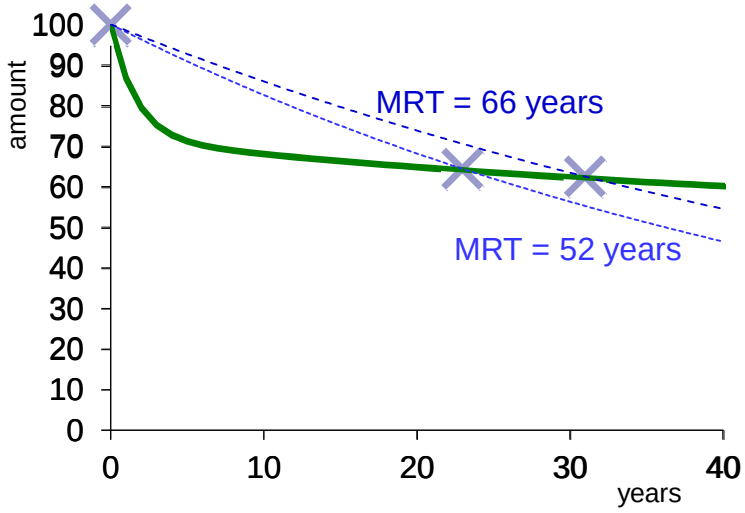


Keel et al. 2006

Problems with FACE, $C_3 \rightarrow C_4$ changes

- Input of „new“, isotopically distinct C
  - Soil C is composed of several pools with organic C in which „old“ C is replaced by „new“ C at different rates.
  - Soil C thus does not have a single turnover time but consists of different pool with different turnover times, decomposition can not be described by **one single**, but **several** exponential functions ($y = A\, e^{-ax} + B\, e^{-bx} + \ldots$).
  - When calculating turnover times according to a one pool model despite it being a multi-pool system, considerable errors can occur depending on the point in time of sampling.





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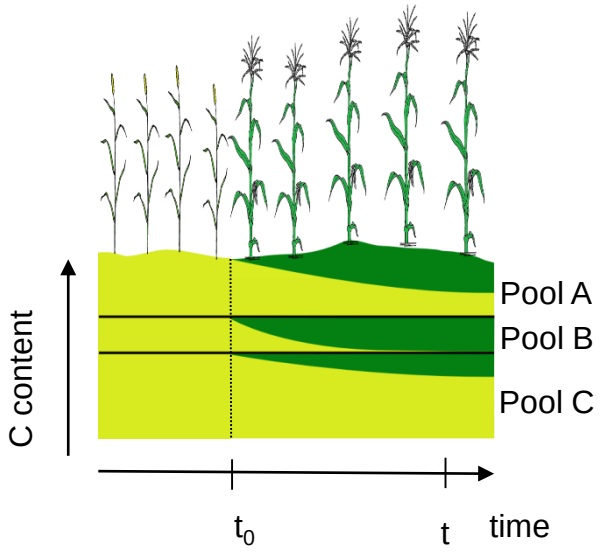
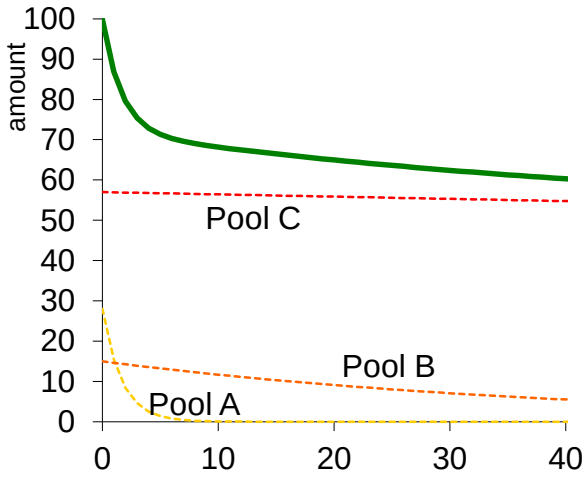
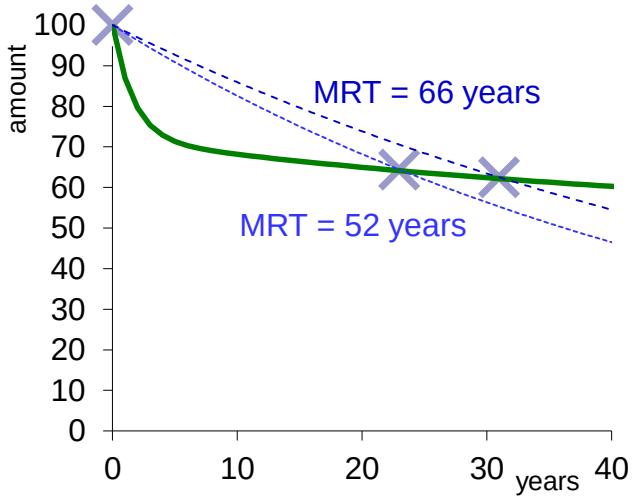
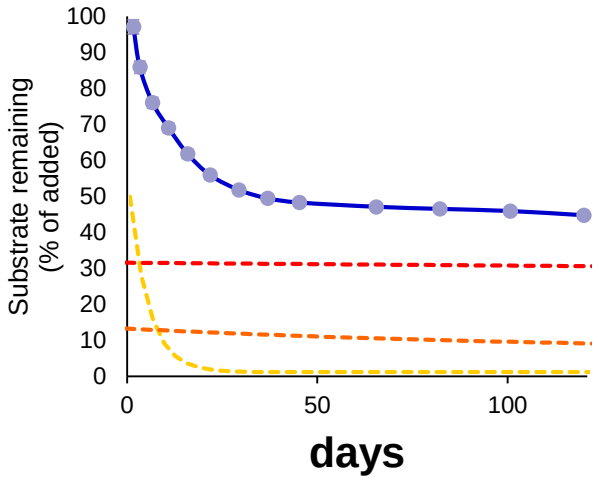






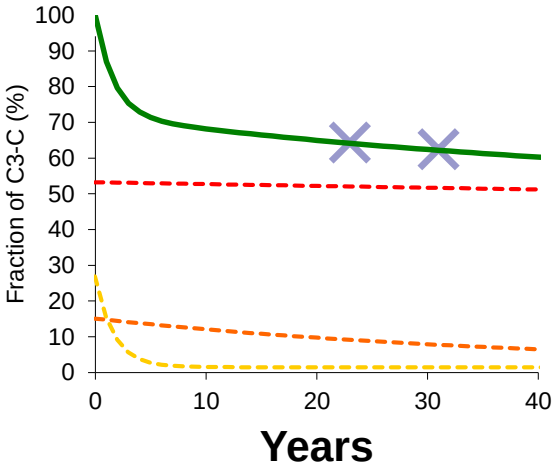
Problems with labelling experiments

- Obviously short term and long-term results do not agree
  - Both assume one-pool models, but there are more pools
  - Substrate exchange among different pools (i.e. substrate recycling) may also be important



- 50% of added leaf litter is decomposed after 100 days

Shahbaz et al. 2016



- > 60% of the carbon in soil is older than 20 years

Basler et al. 2015, John et al. 2005

Problems with labelling experiments

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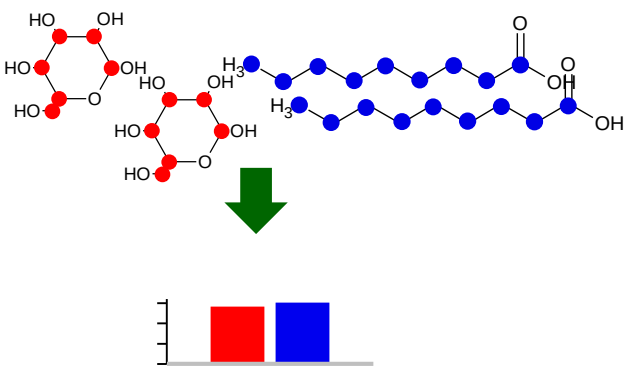

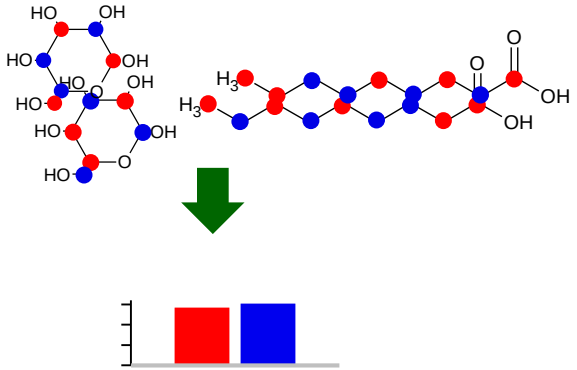

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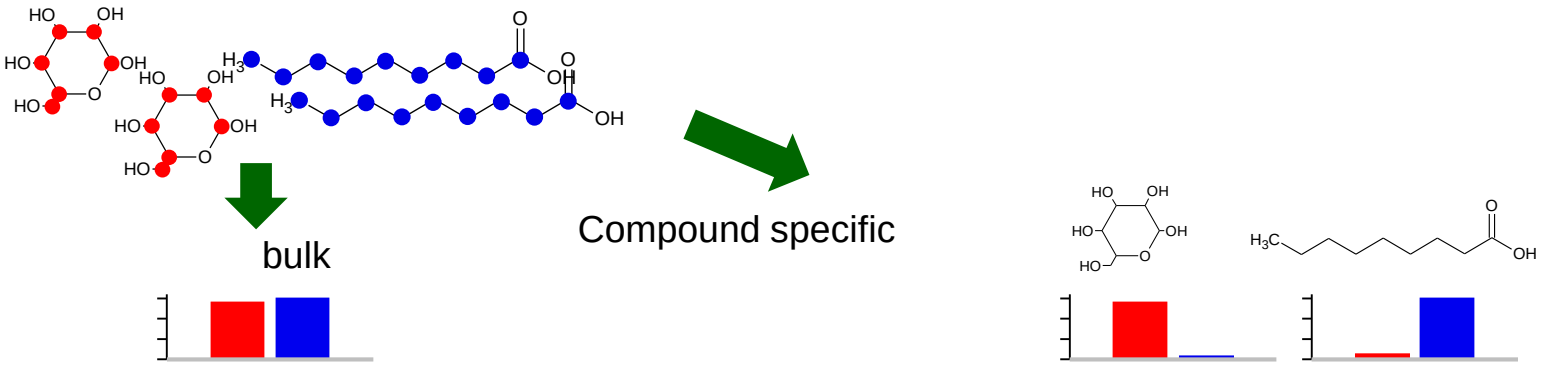

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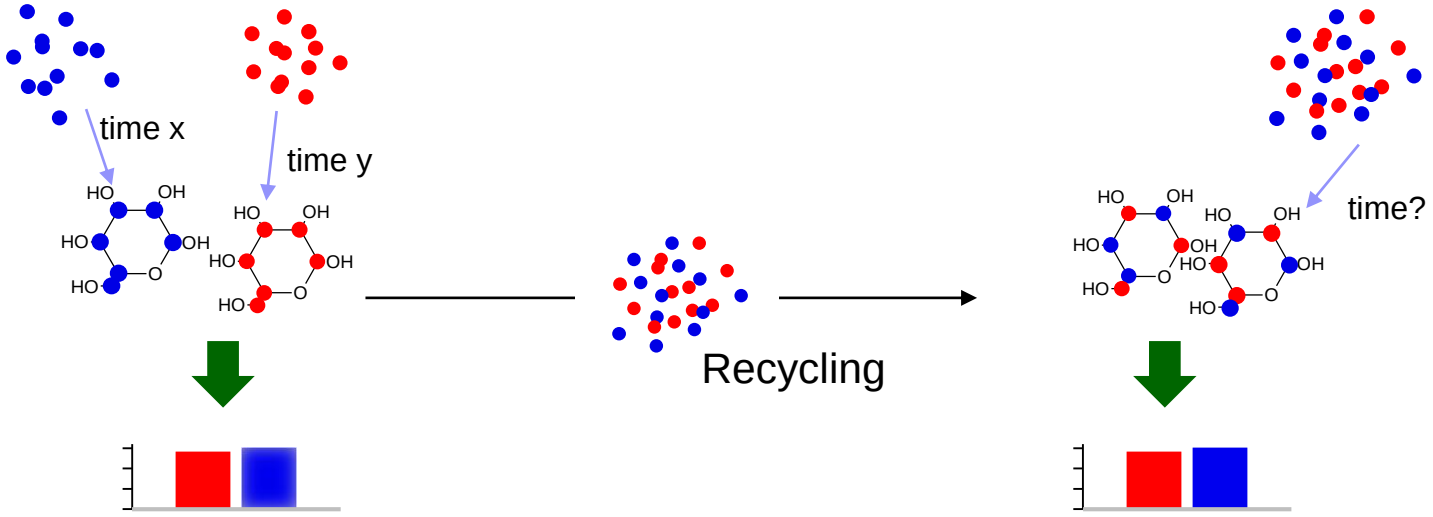
  - Compound specific isotope analysis does not measure MRT of specific molecules, but the MRT of the elements that make up the molecule
    - Time of molecule formation or exchange among pools (i.e. recycling) cannot be detected



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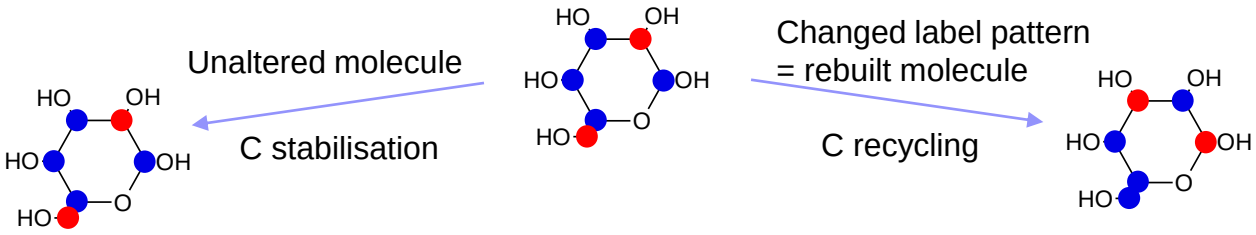
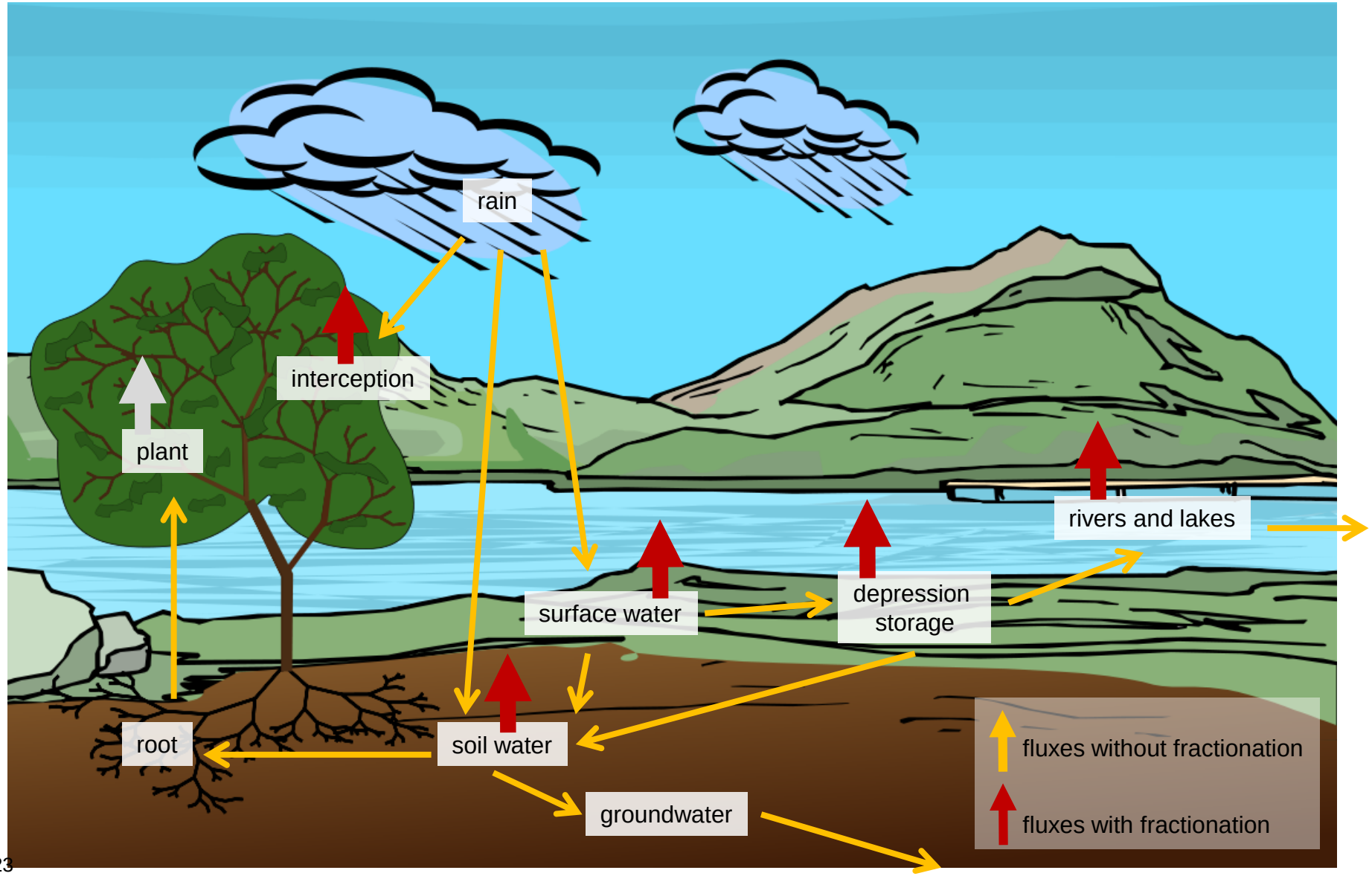
  - Compound specific isotope analysis does not measure MRT of specific molecules, but the MRT of the elements that make up the molecule
    - Time of molecule formation or exchange among pools (i.e. recycling) cannot be detected
  - Recycling of molecules can be detected by using position specific labelling and analysis.
    - Position specific isotope analysis is NOT TRIVIAL

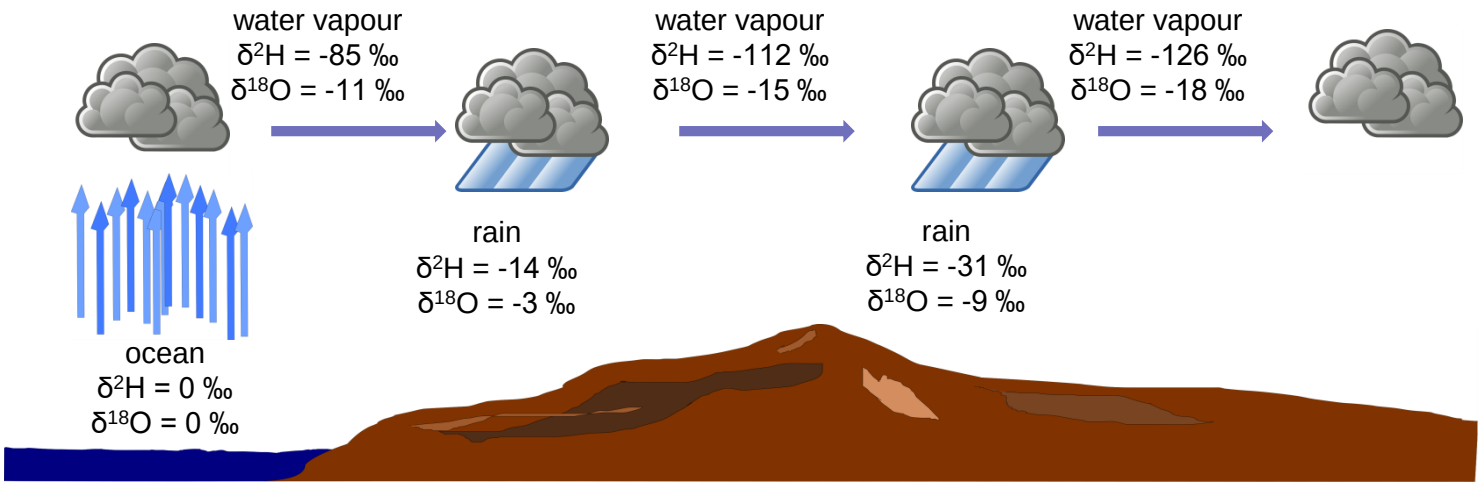


Water cycle in terrestrial ecosystem



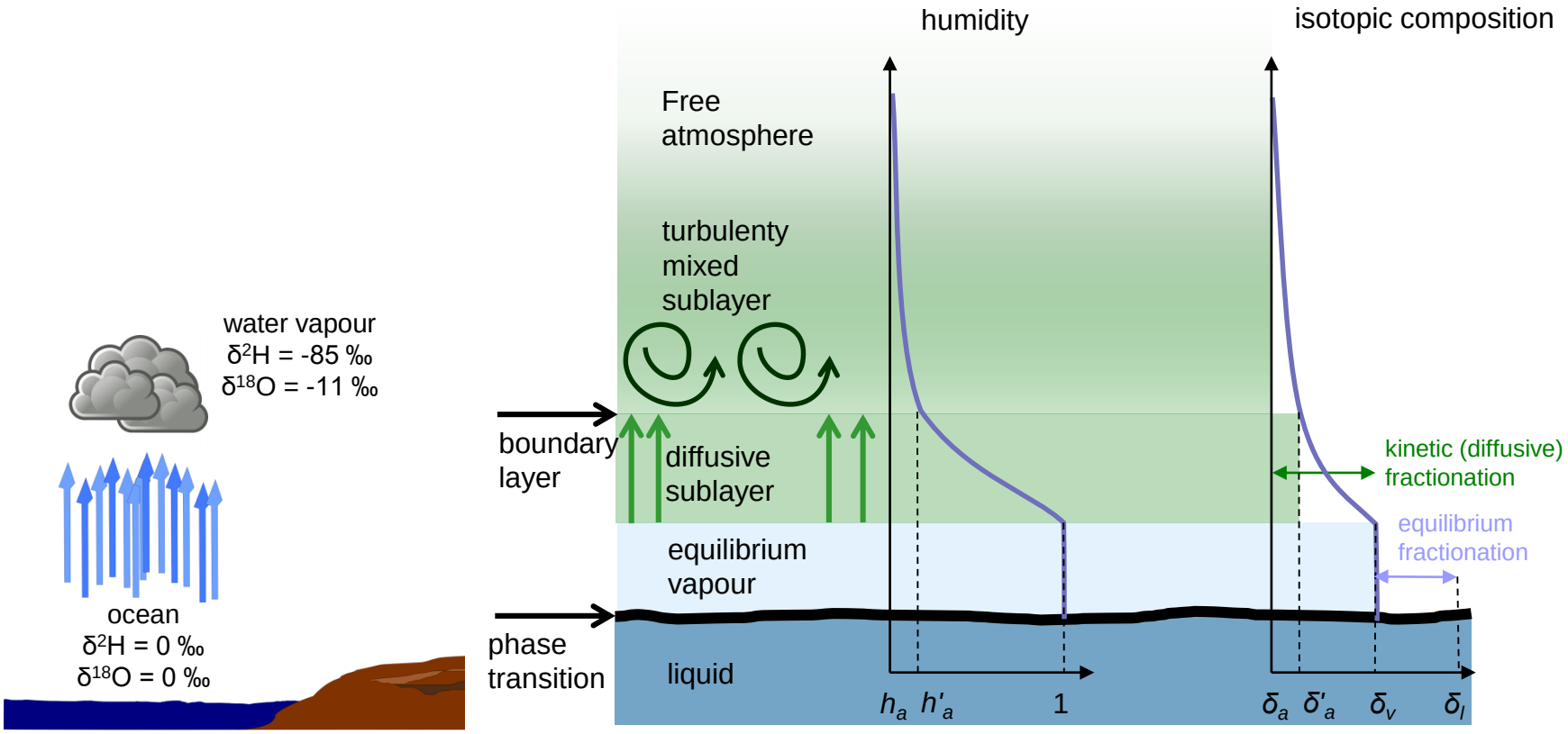
Fractionation during phase transition

- Water evaporation is subject to both equilibrium and kinetic fractionation
  → Water vapour ist depleted in heavy isotopes (light)
- Fromation of rain is subject to equilibrium fractionation,
  heavy isotopes are more abundant in the rain
  → „first rain“ is isotopically enriched (heavy)
  → remaining water vapour becomes more enriched during rain out



Fractionation during phase transition

- Water evaporation is subject to fractionation. This ist due to
  - Equilibrium fractionation and
  - diffusive kinetic fractionation

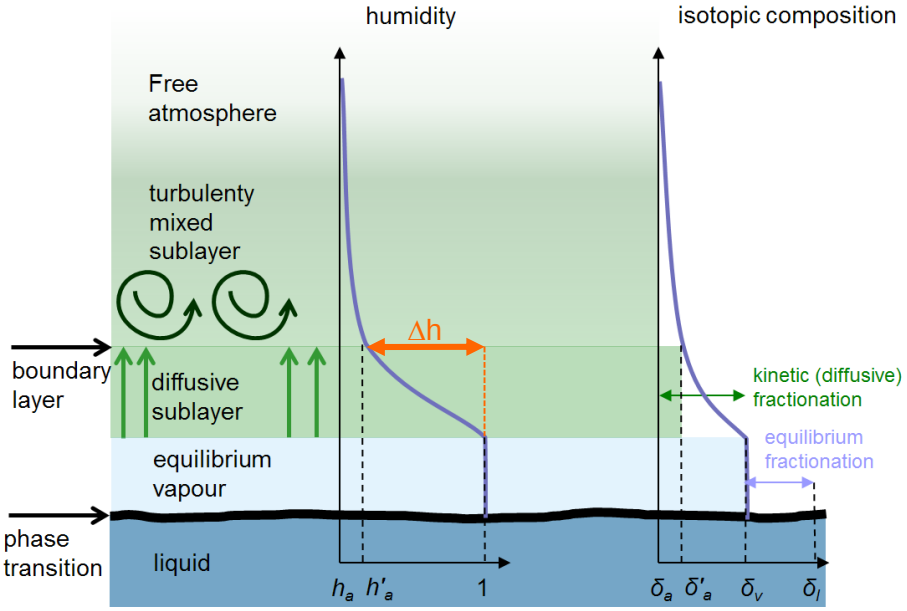


Fractionation during phase transition

- The diffusive kinetic isotope effect depends on the difference in air humidity $\Delta h = 1 - h'_a$ in the diffusion layer
  - Fractionation increases with increasing difference in air humidity Δh

- The equilibrium fractionation of ^{18}O and ^{2}H during evaporation is temperature dependent

t (°C)	$^{2}\varepsilon_{v/l}$ (‰)	$^{18}\varepsilon_{v/l}$ (‰)
0	−101.0	−11.55
5	− 94.8	−11.07
10	− 89.0	−10.60
15	− 83.5	−10.15
20	− 78.4	− 9.71
25	− 73.5	− 9.29
30	− 68.9	− 8.89
35	− 64.6	− 8.49
40	− 60.6	− 8.11



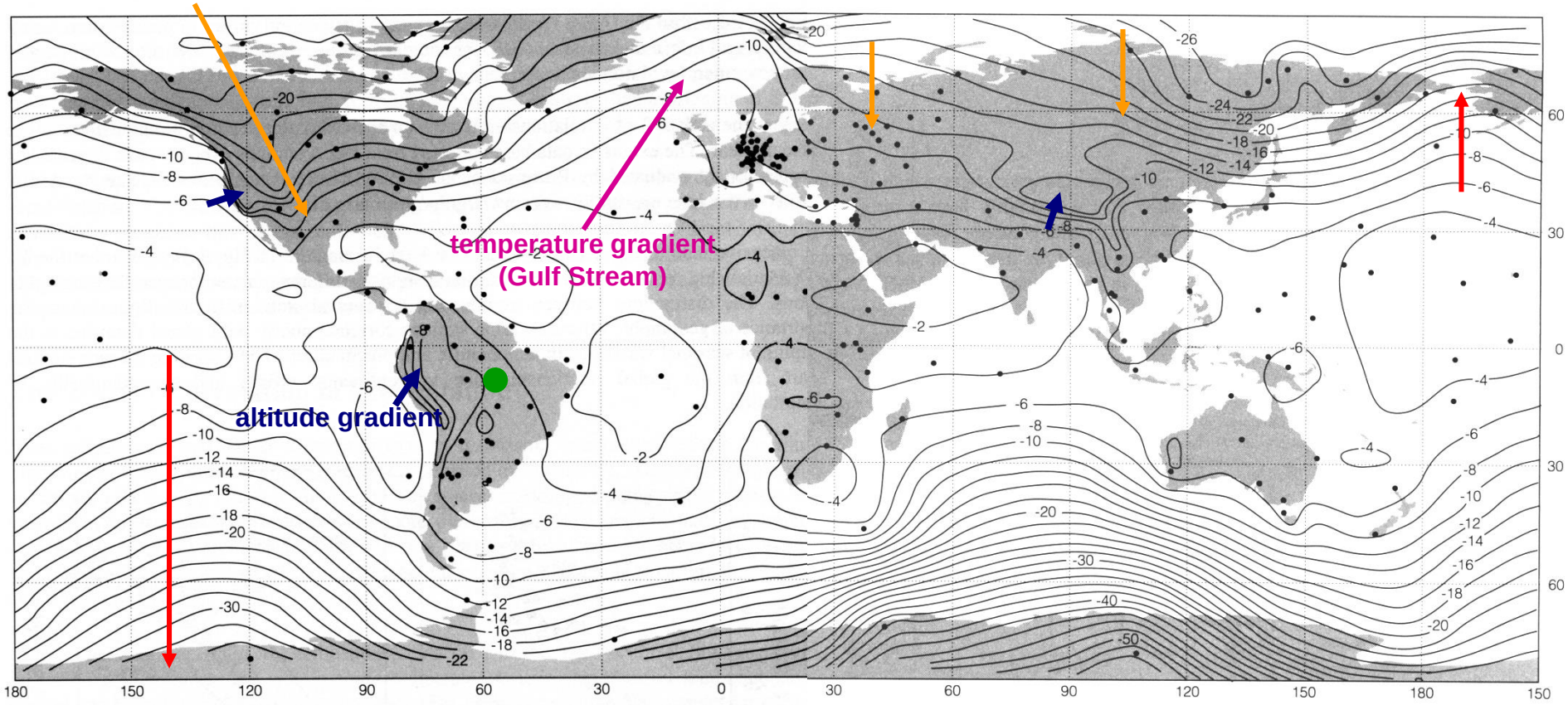
Fractionation during phase transition

- The rain out of a cloud of water vapour with decreasing temperature (and thus decreasing water vapour concentration) corresponds to a Rayleigh-distillation, i.e. loss of enriched water as rain leads to a depletion in the remaining vapour
- Additionally, fractionation increases with decreasing temperature

- Isotopic composition of rain depends on:
  - **Latitude**: decreasing $\delta^{18}O$-values with increasing latitude
  - **Continentality**: decreasing $\delta^{18}O$-values with increasing continentality
  - **Altitude**: decreasing $\delta^{18}O$-values with increasing altitue
  - **Season** (in temperate climates): decreasing $\delta^{18}O$-values in winter
  - **Temperature**: decreasing $\delta^{18}O$-values with decreasing temperature
  - **Amount of rain**: lower $\delta^{18}O$-values in strong rains
    - Isotopically heavy „first rain“ has a decreasing importance with inreasing total amount
    - Less evaporation from raindrops due to high air humidity

$\delta^{18}O$ distribution in precipitation

continentality shifts isotope gradient (isotopically lighter precipitation over the continents)



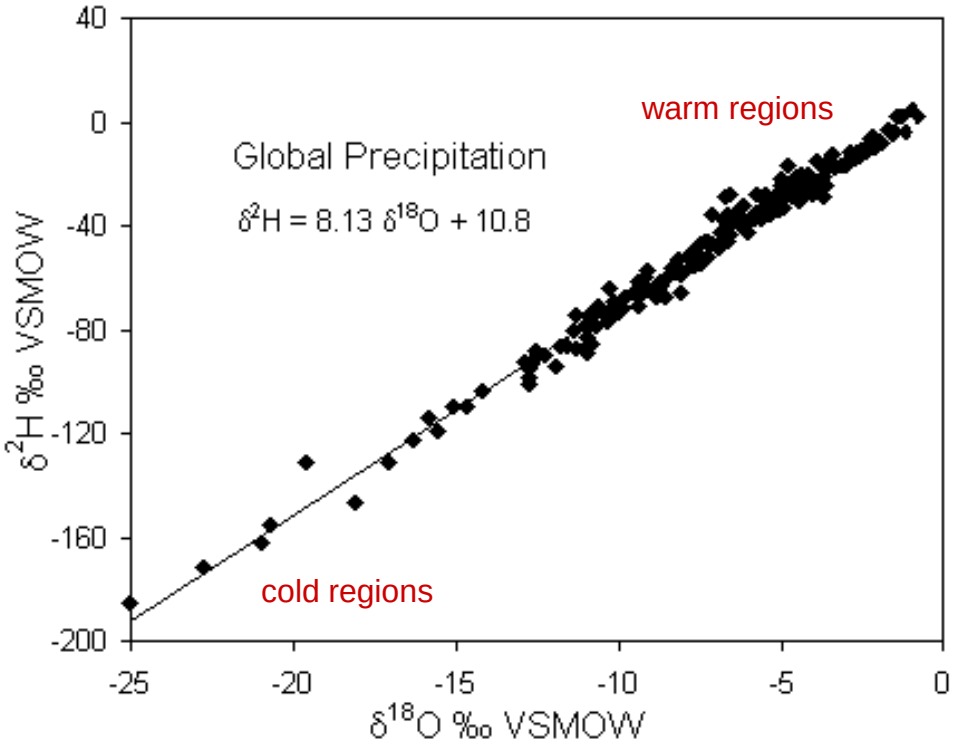
Isotopically ligher precipitation with increasing latitude

Isotopic gradient increases with incrasing latitude

● No gradient over Amazonas bassin indicates strong recycling of water vapour in tropical rain forest

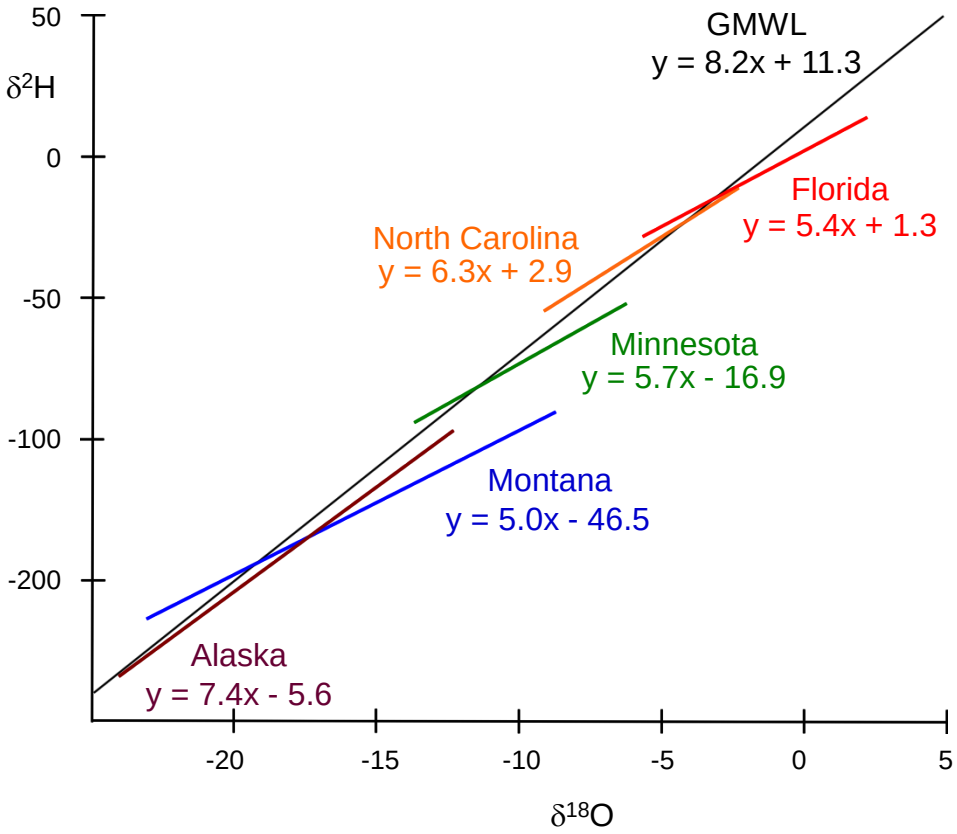
Global Meteoric Water Line

- The relation between $\delta^{18}O$ and $\delta^{2}H$ in surface water (lakes, rivers, precipitation) can globally be described with a linear regression
- This linear relationship is caused by the fact that fractionation for H and O is driven by the same factors
  - temperature
  - air humidity
- In warm regions „heavy“, in cold regions „light“ rain is observed
  - Isotopically heavy „first rain“ falls in warm regions and subsequent rain (in colder regions) is isotopically lighter



Global vs. Local Meteoric Water Line

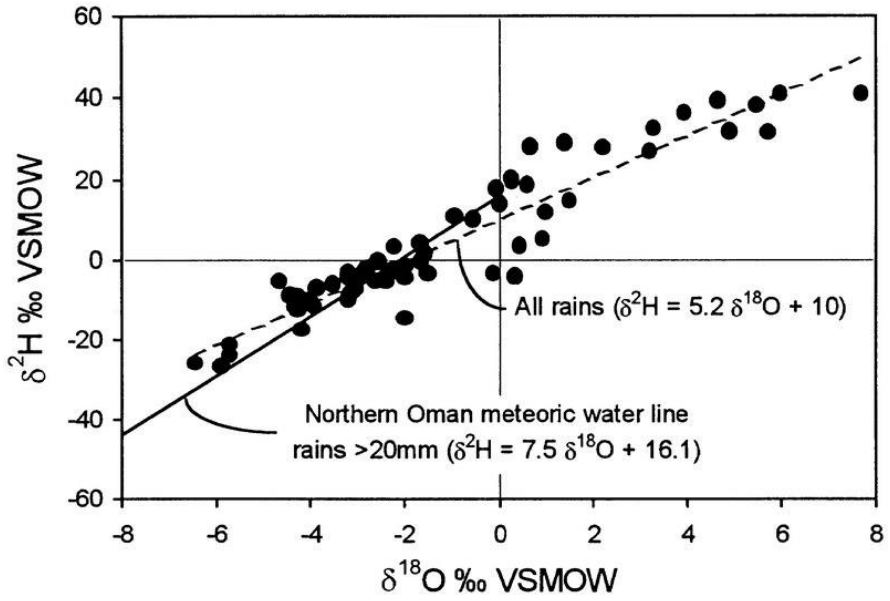
- The GMWL is compiled from regional catchments areas that form local Meteoric Water Lines (LMWLs)
- Separating the points forming the GMWL into local MWLs shows that regional conditions lead to more or less pronounced deviations from GMWL
  - The lower the slope the more important evaporation (with kinetic fractionation) is for a specific LMWL
- Compiling the LMWLs a roof tile pattern emerges



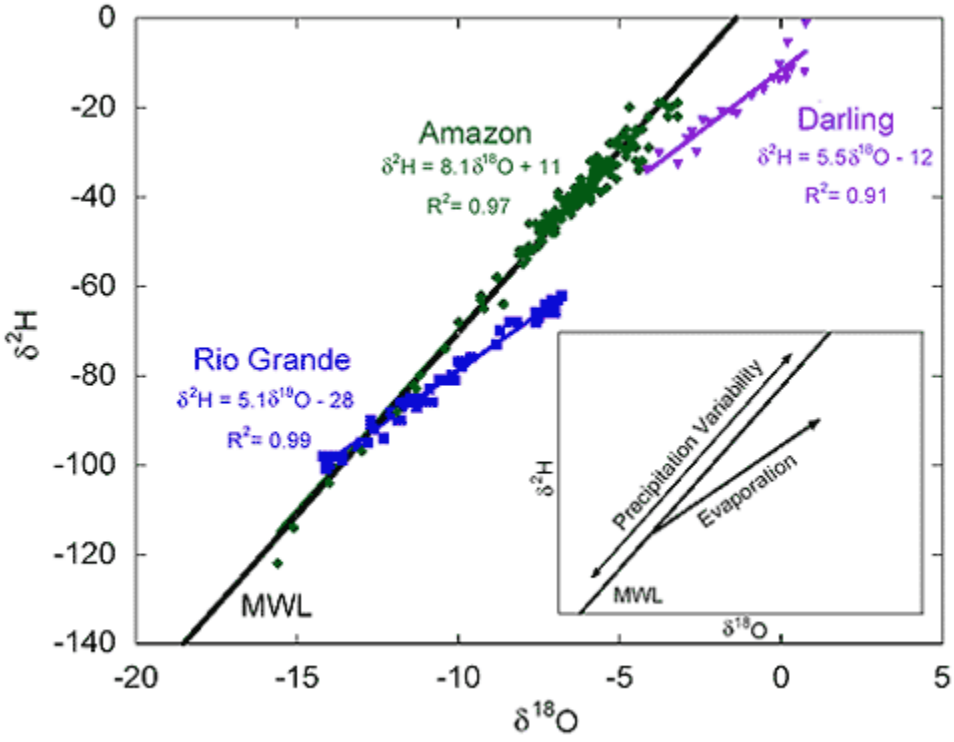
Kendall & Coplen 2001 Hydrol. Processes

Global Meteoric Water Line

- Deviation from the GMWS indicates strong evaporation (e.g. **after** raindrop formation, i.e. condensation)

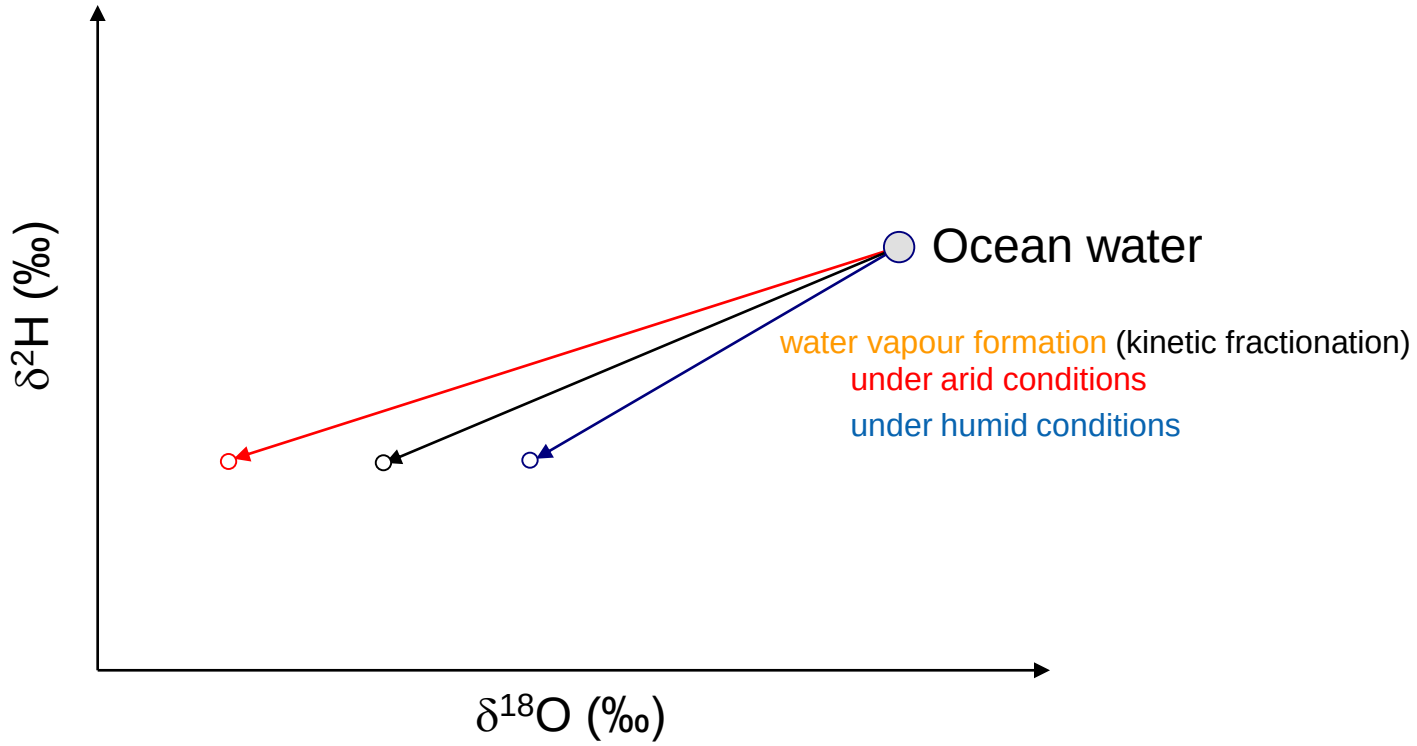


- Similarly, the water of rivers that are subject to strong evaporation can deviate from GMWL



Global Meteoric Water Line

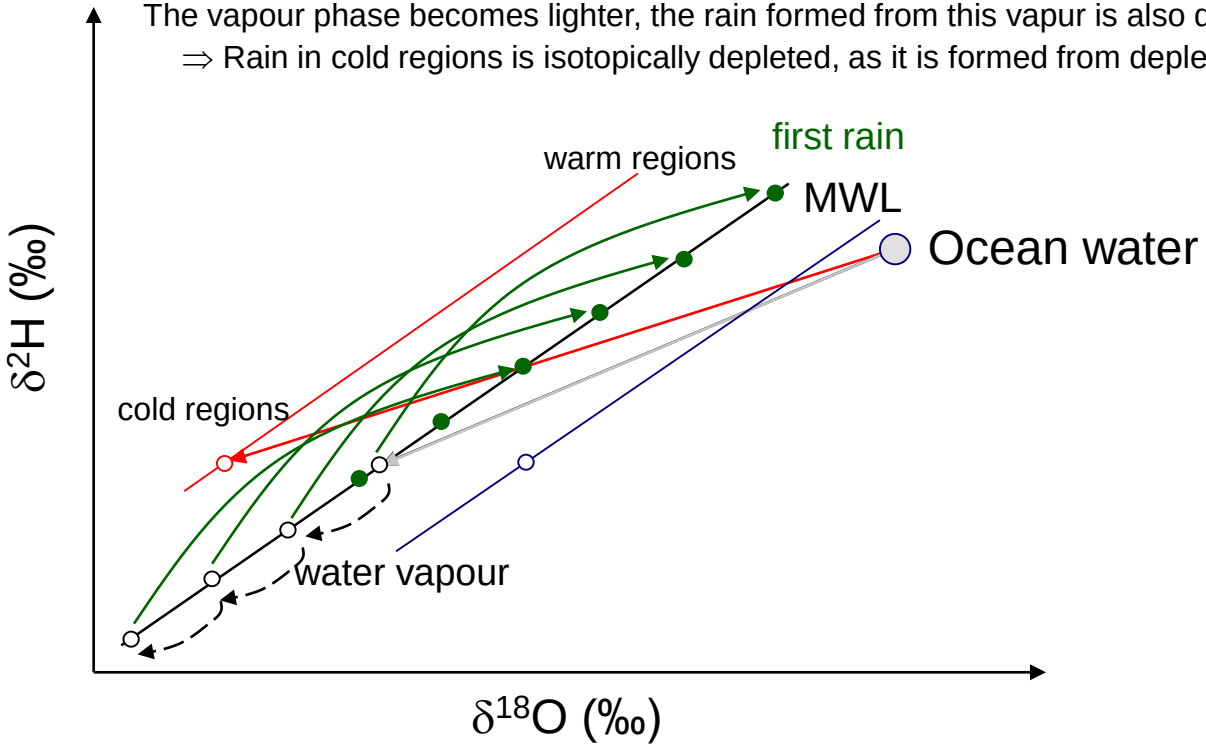
- The factors that influence GMWL: an overview



Global Meteoric Water Line

- The factors that influence GMWL: an overview

condensation (equilibrium fractionation)
Rain is isotopically enriched against the vapour phase
The vapour phase becomes lighter, the rain formed from this vapur is also depleted in ^{13}C
⇒ Rain in cold regions is isotopically depleted, as it is formed from depleted water vapour

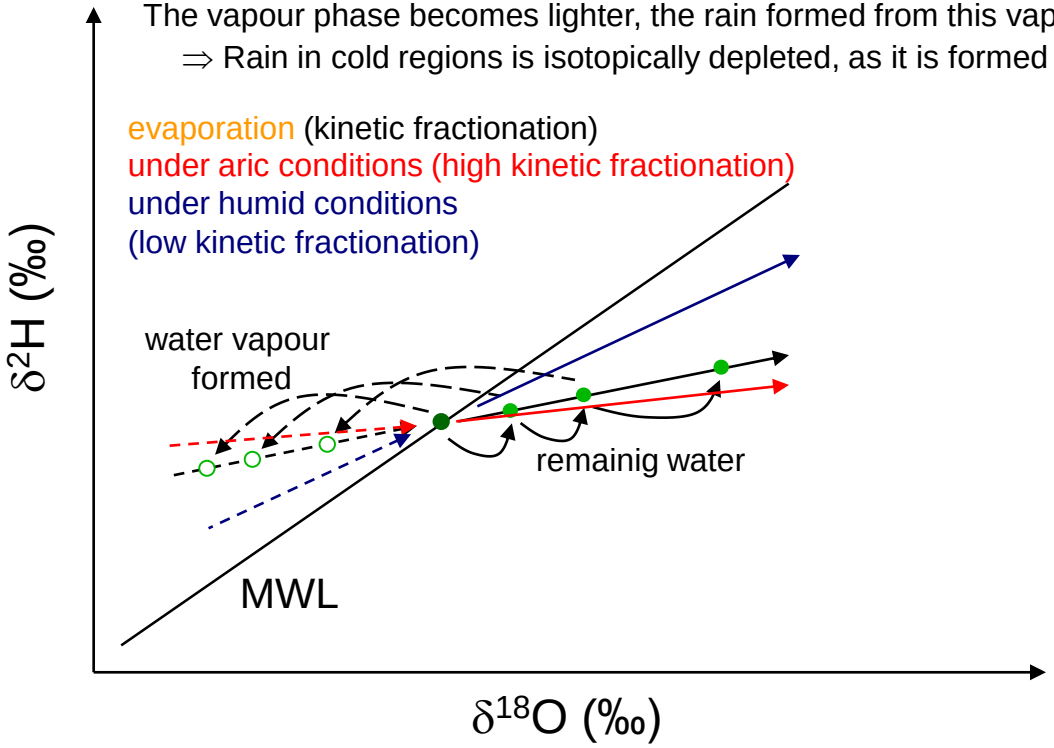


Global Meteoric Water Line

- The factors that influence GMWL: an overview

condensation (equilibrium fractionation)
Rain is isotopically enriched against the vapour phase
The vapour phase becomes lighter, the rain formed from this vapur is also depleted in ^{13}C
⇒ Rain in cold regions is isotopically depleted, as it is formed from depleted water vapour

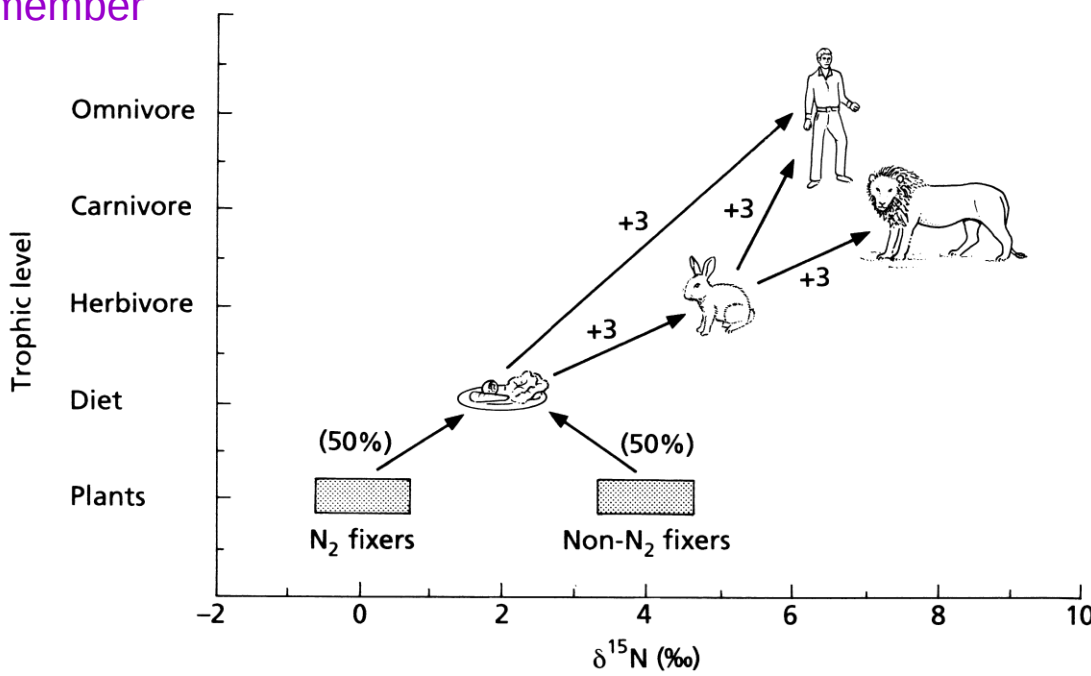
evaporation (kinetic fractionation)
under aric conditions (high kinetic fractionation)
under humid conditions
(low kinetic fractionation)



Food webs

- Isotopes can be used to identify food sources and determine the position of animals in the food web
  - Nitrogen
    - Prey and consumers differ in $\delta^{15}N$ by about 3.4 ‰
    - $\delta^{15}N$ in tissue increase because deamination discriminates against ^{15}N and therefore ^{14}N is increased in excretion (urea, ammonia)

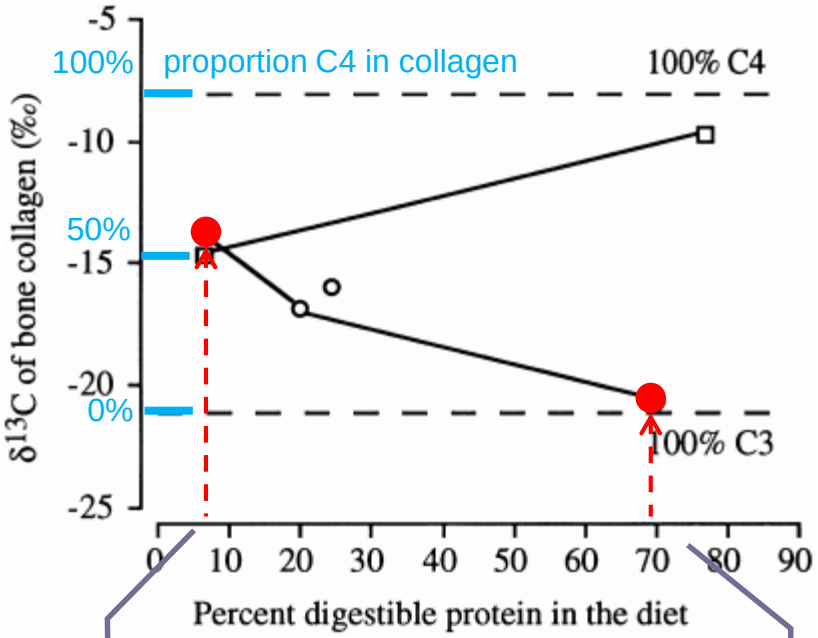
→ The $\delta^{15}N$ value of a food web member is higher for higher positions within the trophic hierachy; consequently the position within the food web can be inferred from $\delta^{15}N$



Food webs

- Pitfalls of food web analysis
  - „isotopic routing“
    Different compounds
    (e.g. proteins, fat, carbohydrates, …)
    are metabolised differently:
    e.g. proteins are preferntially used for tissue formation; carbohydrates are „burned“ (and thus lost) for energy gain
  - The extent of „isotopic routing“ is dependent on food availability

→ The contribution of protein rich food sources for total food supply is overestimated



A protein concentration of < 10% in the diet makes up ~50% of tissue formation

A protein concentration of 70% in the diet makes up 100% of tissue formation

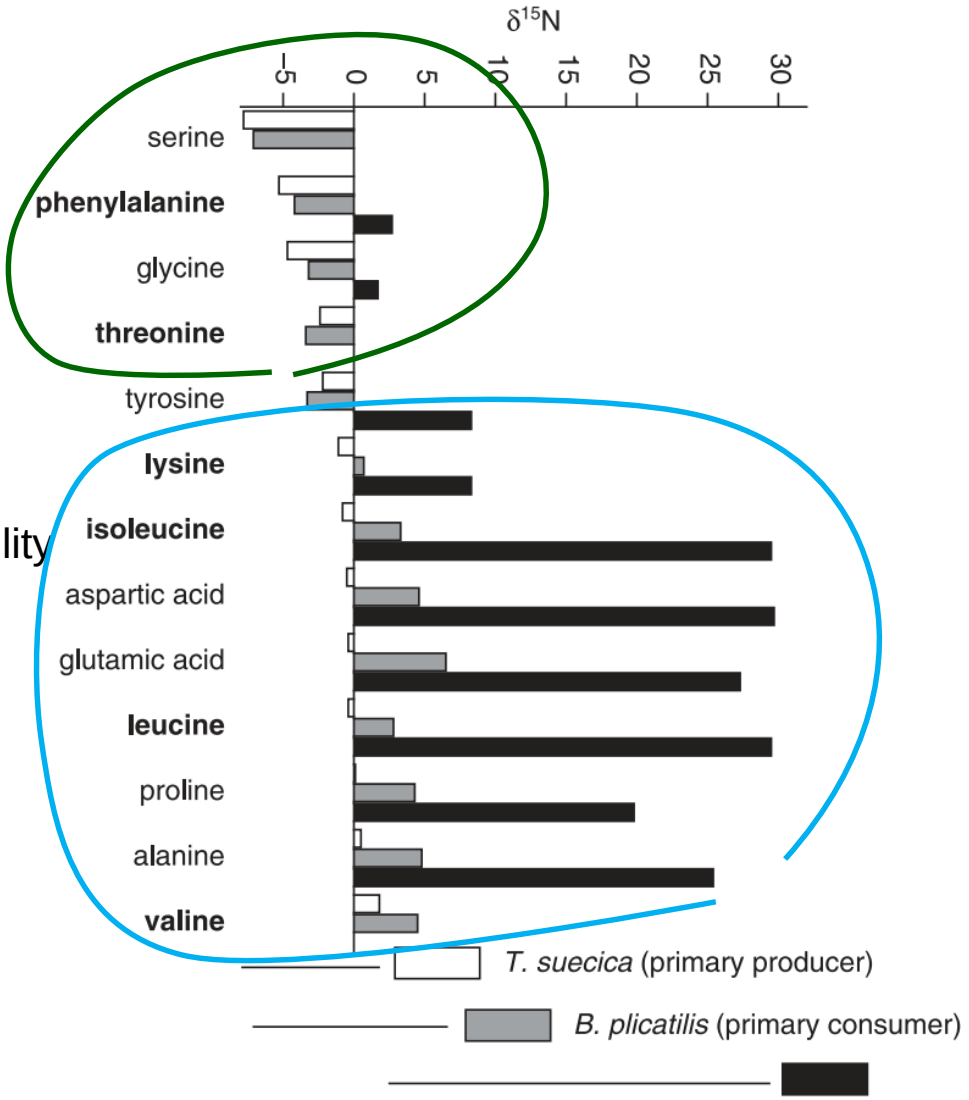
Gannes et al. 1998 Comp. Biochem. Physiol.

Food webs

- Pitfalls of food web analysis
  - Some molecules are incorporated unchanged by the predator,
    → **no isotopic shift**

    others are rebuilt from new material
    → **trophic shift**
  - The importance of direct uptake depends on composition and availability of the food sources

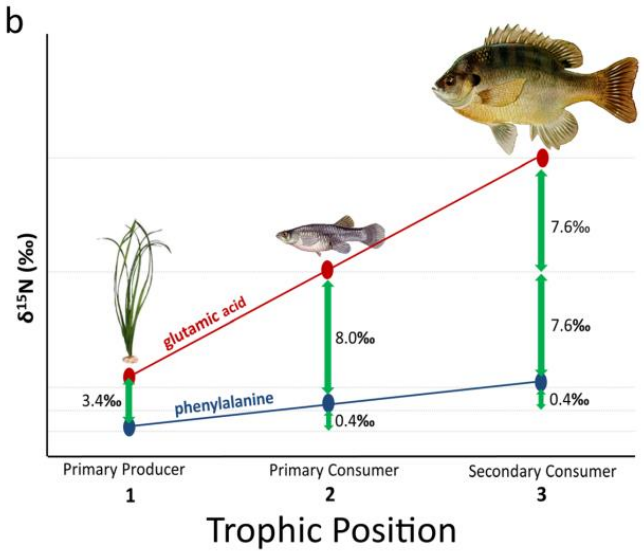
→ Isotopic composition of tissues depends on their chemical nature

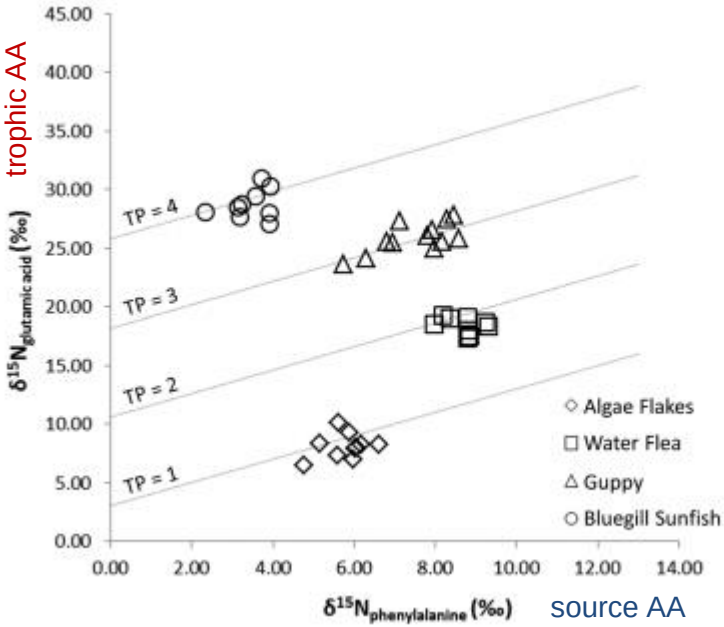


Wolf et al. (2009), Funct. Ecol. 23: 17-26

Food webs

- The different behaviour of newly built amino acids („trophic AA“) and amino acids that are used unaltered („source AA“) can be used to gain a clearer picutre of trophic relationships
  - „Source AA“ reflect the (weighed) isotpic composition of the food source(s), whereas „trophic AA“ indicate the trophic distance between the food web base and the examined group

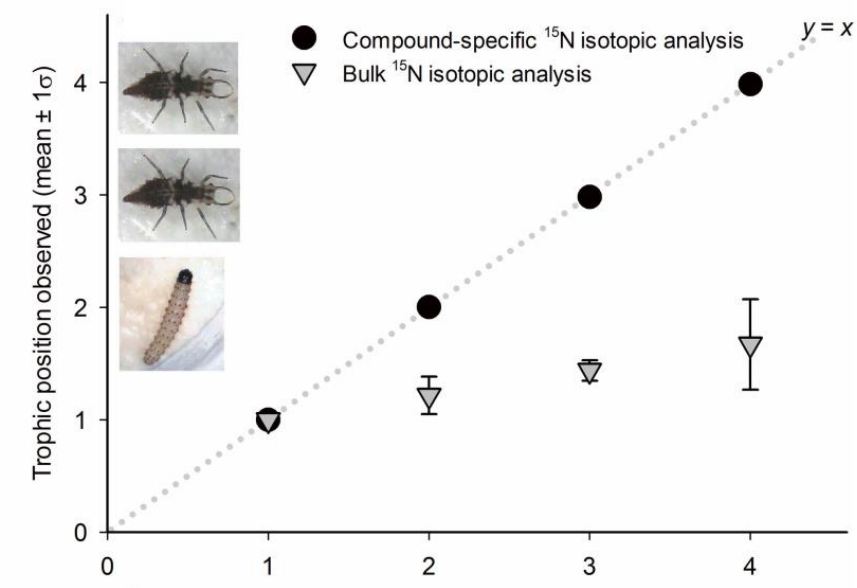




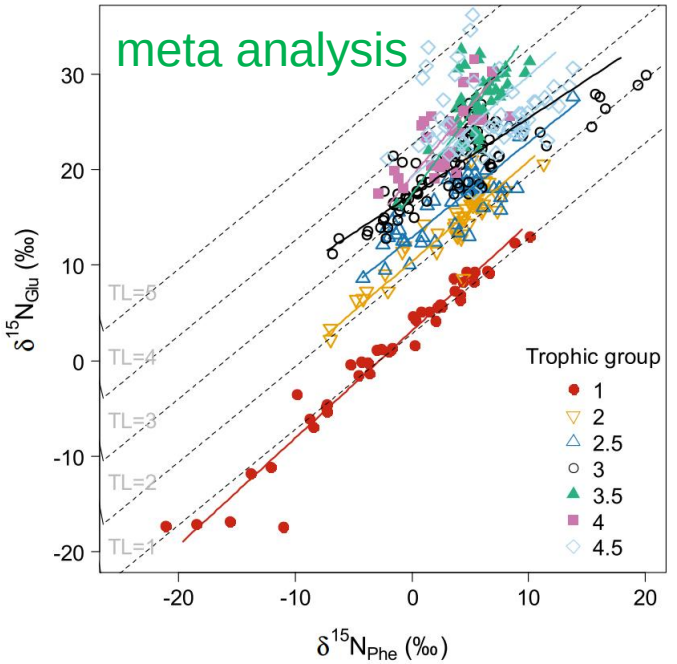
Bowes & Thorp (2015), Ecosphere

Food webs

- The analysis of food web ist more precise when using substance specific isotope analyses, however the necessary effort is much higher
- The comparison of classic approaches to determine trophic positions with isotope analysis shows that the results do not always agree. It remains open which approach yields more accurate results.



Steffan et al. (2013), PLoS one

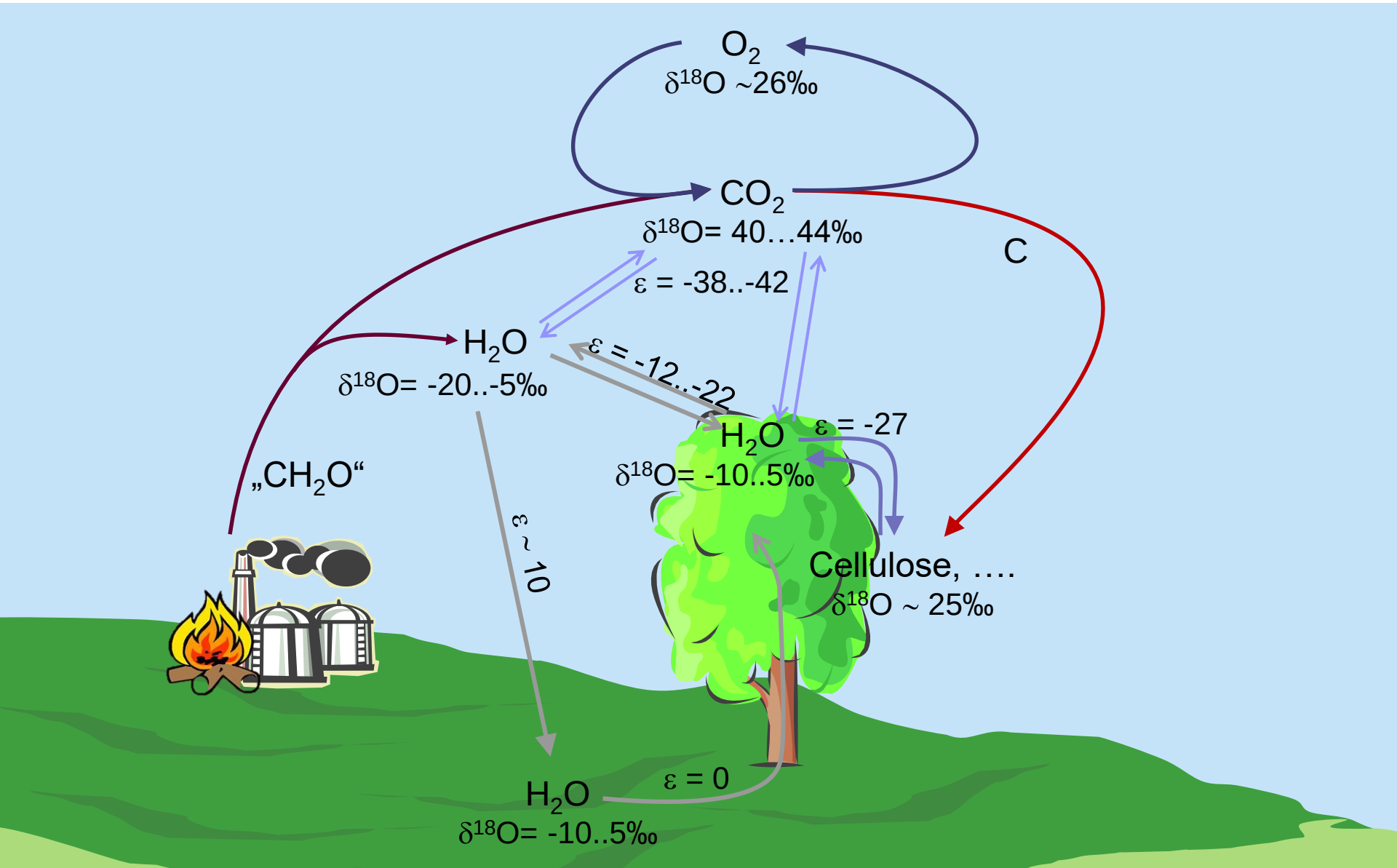


Nielsen et al. (2015), Oecologia

Food webs

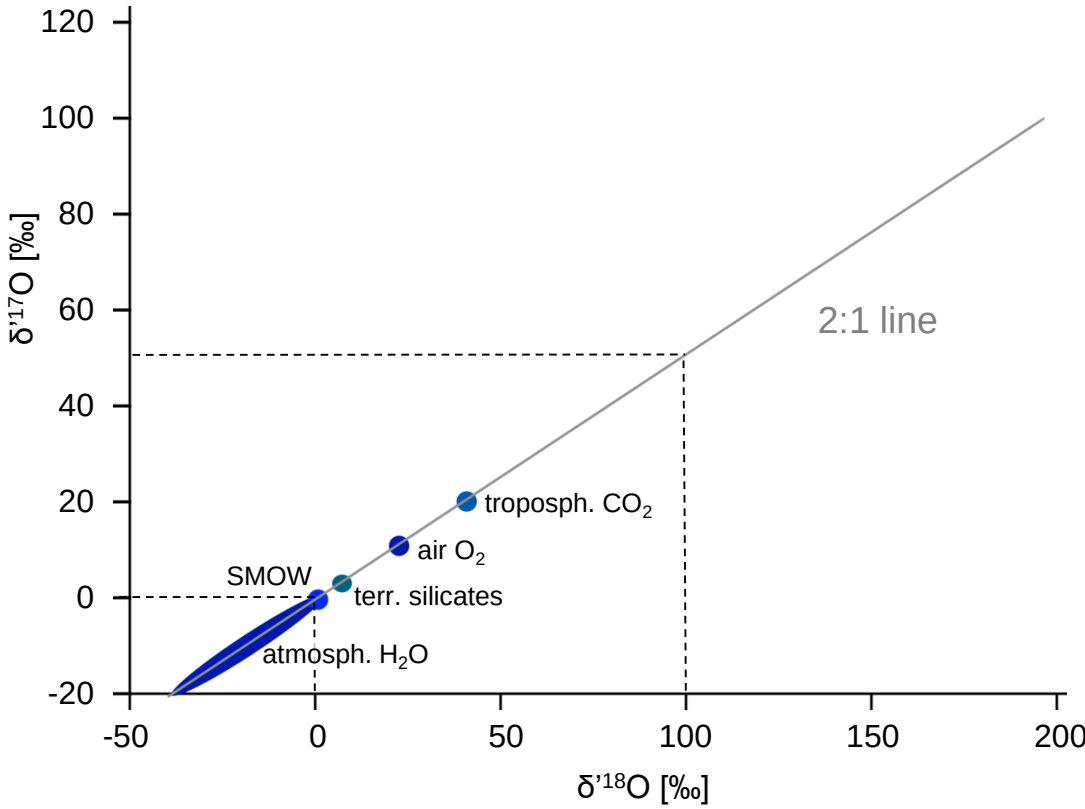
- Pitfalls of food web analysis
  - $\delta^{15}N$ value for starving animals is higher than for well fed animals: Increasing internal N turnover leads to increasing $\delta^{15}N$ because ^{14}N is preferentially excreted
  - The base of a food web (i.e. plants and organic matter) must be identifiable and have consistent isotopic
    - e.g. soil: $\delta^{15}N$ of organic matter increases with increasing soil depth – in what depth are earthworms feeding?
    - Isotopic composition of the base of a food web may be season dependent

Oxygen – Tropospheric Cylce



Oxygen – MIF (mass independent fractionation)

- All fractionation processes we have discussed so far are mass dependent, i.e. size of the fractionation of ^{17}O *vs.* ^{16}O is half the size of the fractionation of ^{18}O *vs.* ^{16}O



Thiemens 2006 Annu. Rev. Earth. Planet. Sci.

Oxygen – MIF (mass independent fractionation)

- All fractionation processes we have discussed so far are mass dependent, i.e. size of the fractionation of ^{17}O *vs.* ^{16}O is half the size of the fractionation of ^{18}O *vs.* ^{16}O
- Certain processes cause mass independent fractionation, e.g. ozone production in the stratosphere

$$O_2 + h\nu \rightarrow \cdot O \cdot + \cdot O \cdot$$

$$\cdot O \cdot + O_2 \rightarrow O_3^*$$

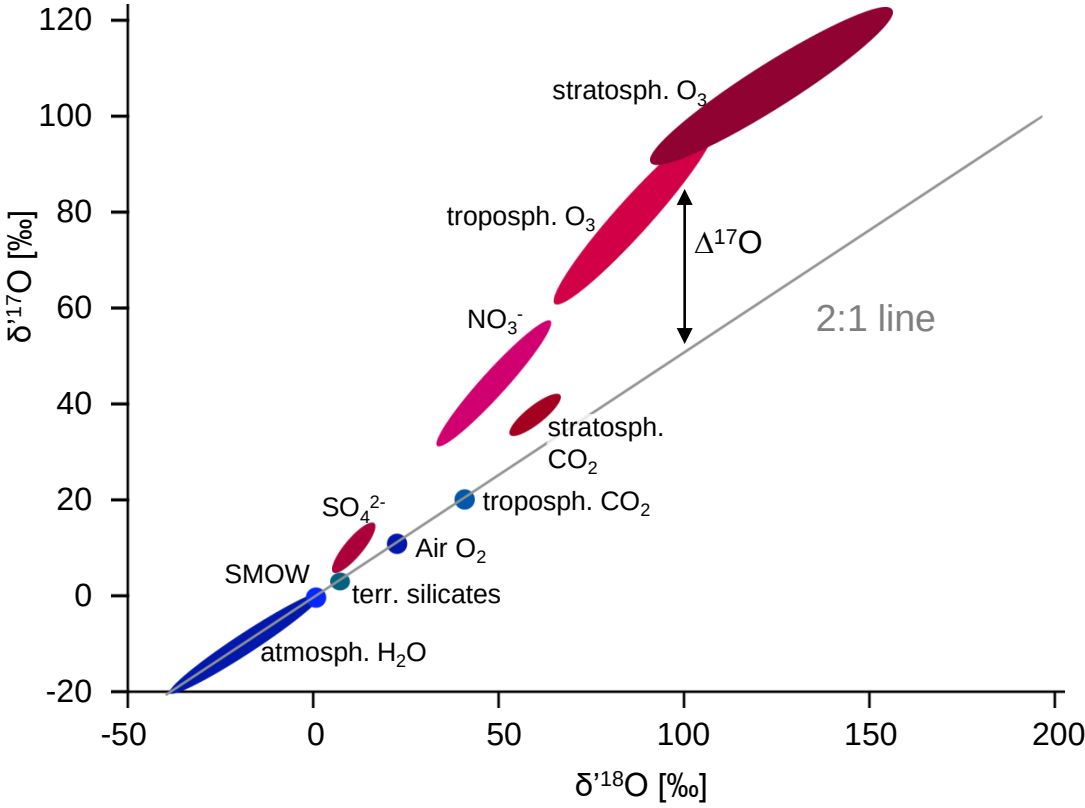
$$O_3^* \xrightarrow{} \cdot O \cdot + O_2$$

$$O_3^* \xrightarrow{M} O_3 + M^*$$

- The frequency of O_3 formation from O_3^* (ozone in an excited, energy rich state) depends on the life time of the O_3^*
  - The longer the life time, the higher the probability that the excess energy can be passed on to a particle M
- Asymmetric molecules (e.g. $^{17}O^{16}O^{16}O$ or $^{18}O^{16}O^{16}O$) are more long lived than the symmetric $^{16}O^{16}O^{16}O$, since more states are available to spread the energy over. This effect is of equal size for $^{17}O^{16}O^{16}O$ and $^{18}O^{16}O^{16}O$
- As a consequence, ozone is strongly enriched in ^{17}O and ^{18}O, the size of enrichment is equal for both ^{17}O and ^{18}O

Oxygen – MIF (mass independent fractionation)

- Stratospheric ozone is strongly enriched in both ^{17}O and ^{18}O to about the same extend (MIF: **M**ass **I**ndependent **F**ractionation), this enrichment is passed on to other gas species, e.g. stratospheric CO_2
  - The extend of the ^{17}O-anomaly ($\Delta^{17}O$ or ^{17}O excess, the deviation from mass dependent fractionation) in atmospheric CO_2 can be used to estimate the contribution of stratospheric (as opposed to biogenic) CO_2. Since CO_2 exchange between stratosphere and atmosphere is known (and constant), $\Delta^{17}O$ can - in principle - be used to calculate the gross primary production (GPP) of the biosphere



Thiemens 2006 Annu. Rev. Earth. Planet. Sci.