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Stable C isotopes in soil organic matter studies

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How do we know that .....

Most of fast food products in US derive from a corn based industry?

How can we investigate...

- The past land use of tropical savannas?

- which waters does a whale swim through?

How can we investigate...

- The migration rote of Snow goose?

- If a sparkling wine is authentic?

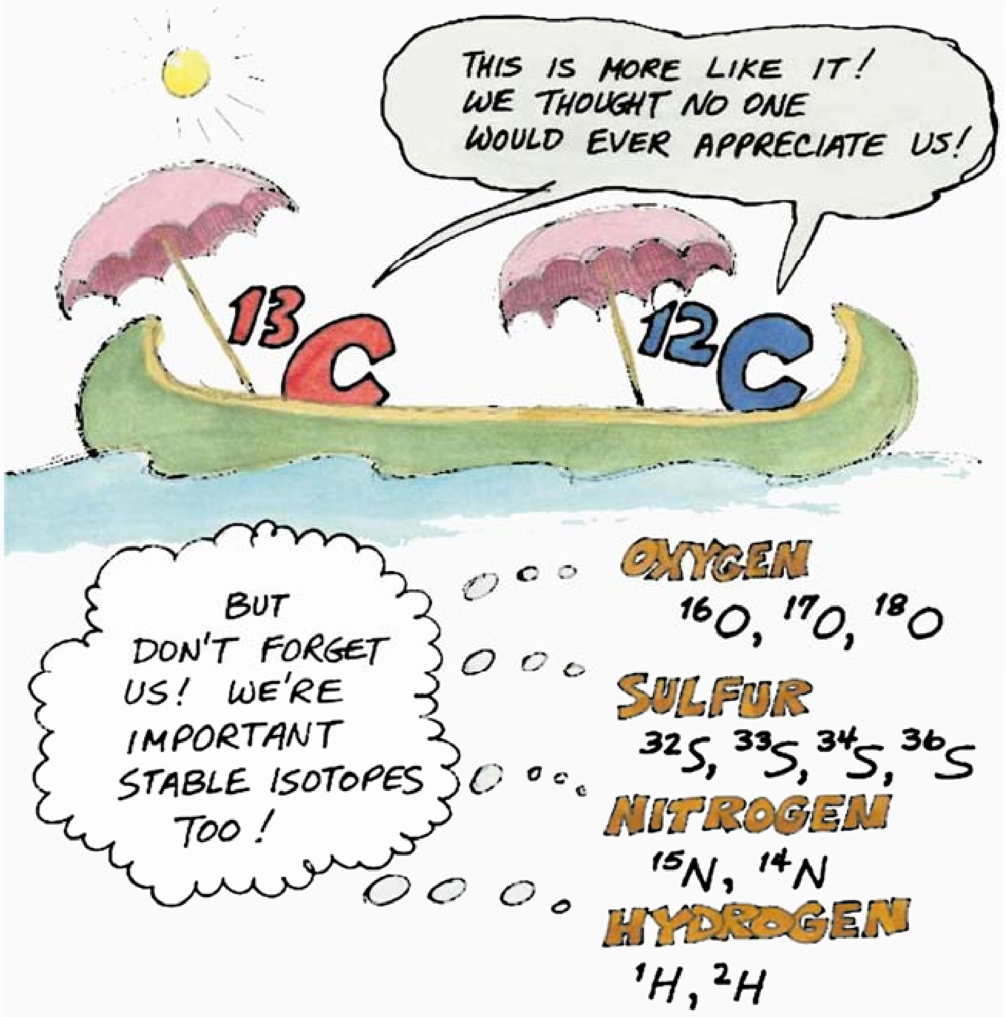
How can we reconstruct...

- The climate of the past?

- The diet of ancient civilization?

Isotopes are a powerful tool in ecology and environmental science research

In particular large progress has been made in the understanding and quantification of soil organic carbon dynamics using isotope methods



Graphic from Fry, 2006

Outline:

- ✓ Isotopes, units and standards
- ✓ Stable isotope measurement systems
- ✓ Natural abundance of stable C isotopes
- ✓ Fractionation processes
- ✓ Mass balance approach
- ✓ Rare isotope enrichment studies

<u>Studies in SOC dynamics:</u>

Partitioning CO_2 fluxes and SOC pools

Tracing C dynamics in plant and soil

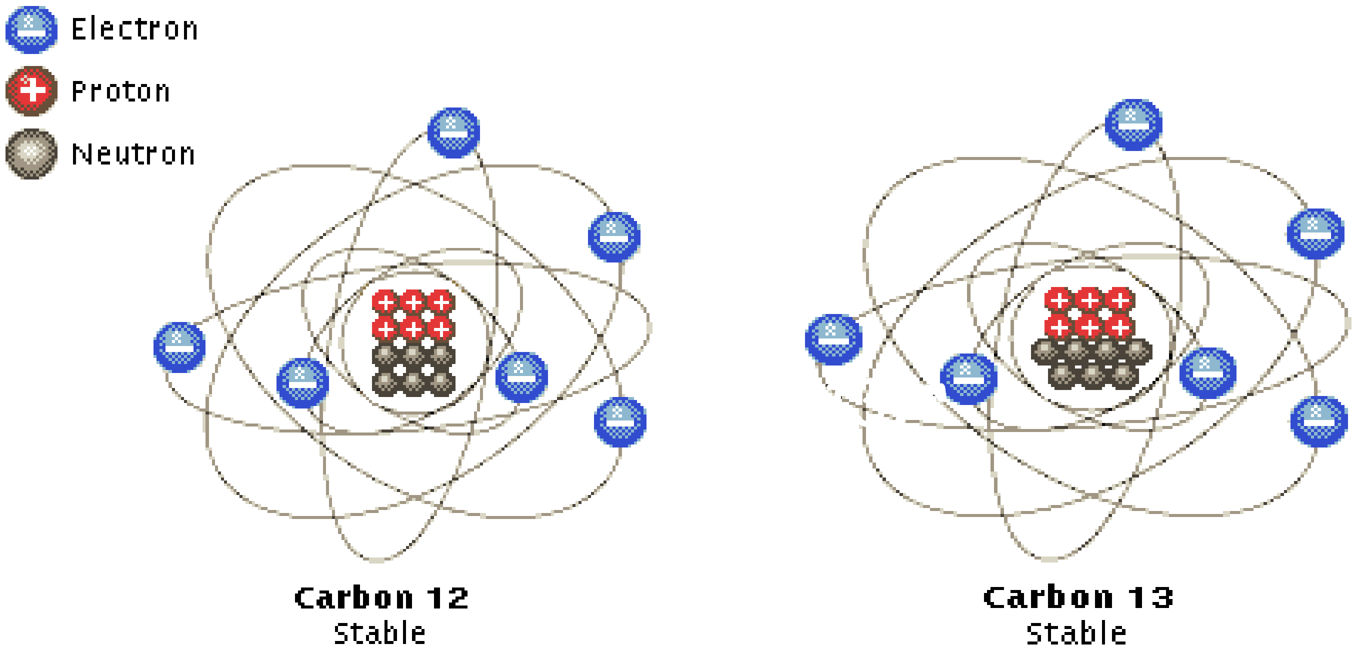
What is an Isotope?

- “**ISOTOPE**”: from *iso* = same and *topos*= place, i.e. share the same location on the periodic table

- **Isotopes** of a chemical element are atoms whose nuclei have the same atomic number but different atomic mass (i.e. different number of neutrons)

- *Isotope vs. nuclide generates confusion!* A **nuclide** is any particular atomic nucleus with a specific atomic number *Z* and mass number *A*; it is equivalently an atomic nucleus with a specific number of protons and neutrons. Collectively, all the isotopes of all the elements form the set of *nuclides*. The distinction between the terms *isotope* and *nuclide* has somewhat blurred, and they are often used interchangeably. *Isotope* is best used when referring to several different nuclides of the same element; *nuclide* is more generic and is used when referencing only one nucleus or several nuclei of different elements.

Stable C Isotopes



6	Protons	⟵ *Same* ⟶	6	Protons
6	Neutrons		7	Neutrons
12			13	

Periodic Table of the Elements*

Atomic Number — 3
Symbol — Li
Atomic Mass** — 6.941

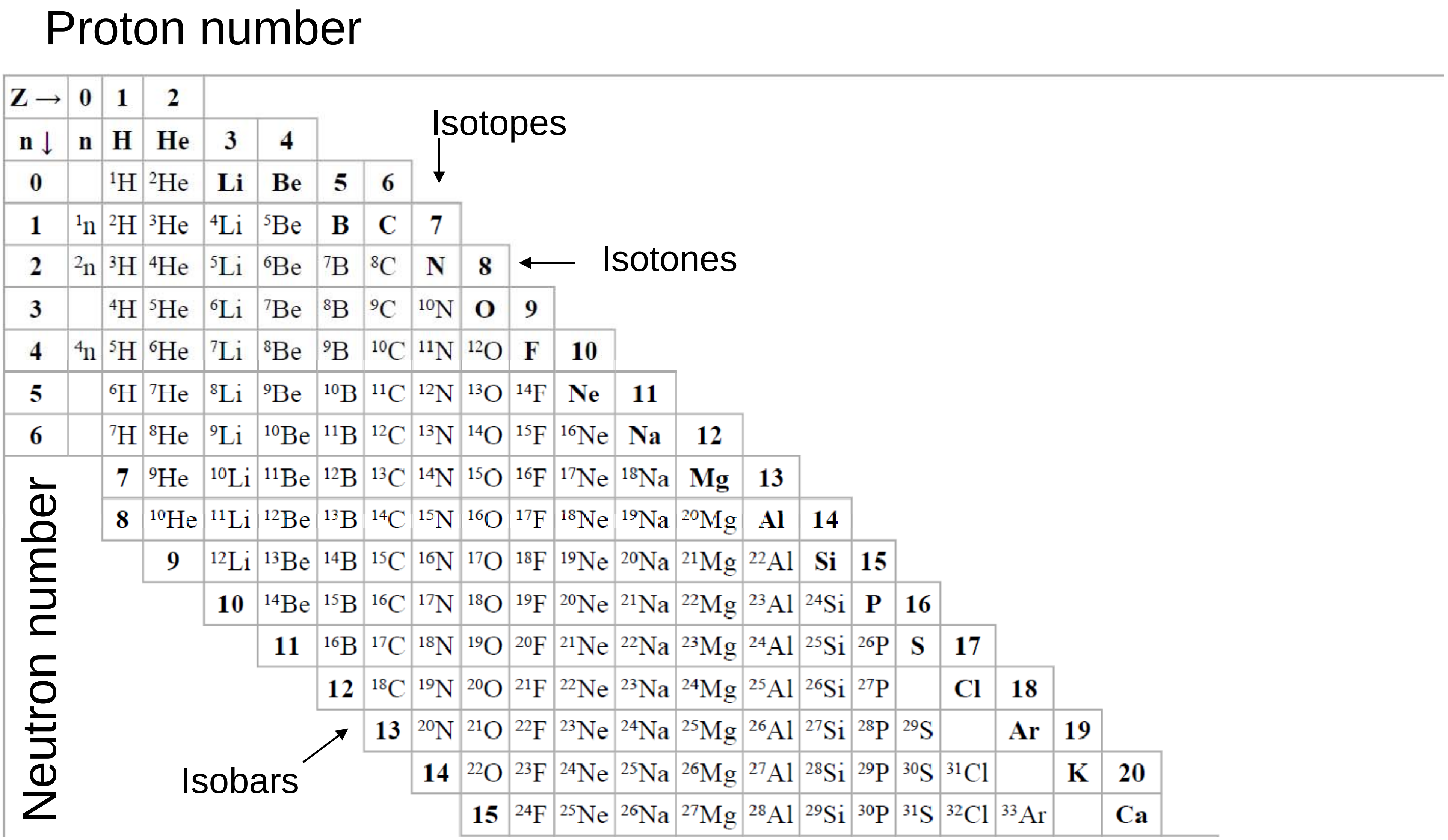
I A	II A	III B	IV B	V B	VI B	VII B	VIII B			I B	II B	III A	IV A	V A	VI A	VII A	VIII A
1 H 1.0079																	2 He 4.0026
3 Li 6.941	4 Be 9.0122											5 B 10.811	6 C 12.011	7 N 14.007	8 O 15.999	9 F 18.998	10 Ne 20.180
11 Na 22.990	12 Mg 24.305											13 Al 26.982	14 Si 28.086	15 P 30.974	16 S 32.066	17 Cl 35.453	18 Ar 39.948
19 K 39.098	20 Ca 40.078	21 Sc 44.956	22 Ti 47.867	23 V 50.942	24 Cr 51.996	25 Mn 54.938	26 Fe 55.845	27 Co 58.933	28 Ni 58.693	29 Cu 63.546	30 Zn 65.39	31 Ga 69.723	32 Ge 72.61	33 As 74.922	34 Se 78.96	35 Br 79.904	36 Kr 83.80
37 Rb 85.468	38 Sr 87.62	39 Y 88.906	40 Zr 91.224	41 Nb 92.906	42 Mo 95.94	43 Tc (98)	44 Ru 101.07	45 Rh 102.906	46 Pd 106.42	47 Ag 107.87	48 Cd 112.41	49 In 114.82	50 Sn 118.71	51 Sb 121.76	52 Te 127.62	53 I 126.90	54 Xe 131.29
55 Cs 132.90	56 Ba 137.33	57 †La 138.90	72 Hf 178.49	73 Ta 180.95	74 W 183.84	75 Re 186.21	76 Os 190.23	77 Ir 192.22	78 Pt 195.08	79 Au 196.97	80 Hg 200.59	81 Tl 204.38	82 Pb 207.2	83 Bi 208.98	84 Po (209)	85 At (210)	86 Rn (222)
87 Fr (223)	88 Ra (226)	89 ‡Ac (227)	104 Rf (261)	105 Ha (262)													

† Lanthanides	58 Ce 140.12	59 Pr 140.91	60 Nd 144.24	61 Pm (145)	62 Sm 150.36	63 Eu 151.96	64 Gd 157.25	65 Tb 158.92	66 Dy 162.50	67 Ho 164.93	68 Er 167.26	69 Tm 168.93	70 Yb 173.04	71 Lu 174.97
‡ Actinides	90 Th 232.04	91 Pa 231.04	92 U 238.03	93 Np (237)	94 Pu (244)	95 Am (243)	96 Cm (247)	97 Bk (247)	98 Cf (251)	99 Es (252)	100 Fm (257)	101 Md (258)	102 No (259)	103 Lw (262)

* Data adapted from Lide, D.R., Editor (1995) *CRC Handbook of Chemistry and Physics*, 76th Edition. CRC Press, Boca Raton, Florida.

** Atomic mass values in parentheses denote the atomic mass of the most stable isotope of those elements too unstable for the determination of a standard atomic mass.

Nuclide chart



Z →	**0**	**1**	**2**																		
n ↓	**n**	**H**	**He**	**3**	**4**																
0		^{1}H	^{2}He	**Li**	**Be**	**5**	**6**														
1	^{1}n	^{2}H	^{3}He	^{4}Li	^{5}Be	**B**	**C**	**7**													
2	^{2}n	^{3}H	^{4}He	^{5}Li	^{6}Be	^{7}B	^{8}C	**N**	**8**												
3		^{4}H	^{5}He	^{6}Li	^{7}Be	^{8}B	^{9}C	^{10}N	**O**	**9**											
4	^{4}n	^{5}H	^{6}He	^{7}Li	^{8}Be	^{9}B	^{10}C	^{11}N	^{12}O	**F**	**10**										
5		^{6}H	^{7}He	^{8}Li	^{9}Be	^{10}B	^{11}C	^{12}N	^{13}O	^{14}F	**Ne**	**11**									
6		^{7}H	^{8}He	^{9}Li	^{10}Be	^{11}B	^{12}C	^{13}N	^{14}O	^{15}F	^{16}Ne	**Na**	**12**								
		7	^{9}He	^{10}Li	^{11}Be	^{12}B	^{13}C	^{14}N	^{15}O	^{16}F	^{17}Ne	^{18}Na	**Mg**	**13**							
		8	^{10}He	^{11}Li	^{12}Be	^{13}B	^{14}C	^{15}N	^{16}O	^{17}F	^{18}Ne	^{19}Na	^{20}Mg	**Al**	**14**						
			9	^{12}Li	^{13}Be	^{14}B	^{15}C	^{16}N	^{17}O	^{18}F	^{19}Ne	^{20}Na	^{21}Mg	^{22}Al	**Si**	**15**					
				10	^{14}Be	^{15}B	^{16}C	^{17}N	^{18}O	^{19}F	^{20}Ne	^{21}Na	^{22}Mg	^{23}Al	^{24}Si	**P**	**16**				
					11	^{16}B	^{17}C	^{18}N	^{19}O	^{20}F	^{21}Ne	^{22}Na	^{23}Mg	^{24}Al	^{25}Si	^{26}P	**S**	**17**			
						12	^{18}C	^{19}N	^{20}O	^{21}F	^{22}Ne	^{23}Na	^{24}Mg	^{25}Al	^{26}Si	^{27}P		**Cl**	**18**		
							13	^{20}N	^{21}O	^{22}F	^{23}Ne	^{24}Na	^{25}Mg	^{26}Al	^{27}Si	^{28}P	^{29}S		**Ar**	**19**	
								14	^{22}O	^{23}F	^{24}Ne	^{25}Na	^{26}Mg	^{27}Al	^{28}Si	^{29}P	^{30}S	^{31}Cl		**K**	**20**
									15	^{24}F	^{25}Ne	^{26}Na	^{27}Mg	^{28}Al	^{29}Si	^{30}P	^{31}S	^{32}Cl	^{33}Ar		**Ca**

Types of Isotopes

- Isotopes of one element have different neutron/proton ratios. This affects their **nuclear stability**.
  → presence of either stable isotopes with stable nuclei or unstable isotopes (subject to nuclear decay)
- Stable isotopes
  - One overwhelmingly abundant isotope + other of minor abundance
  - However, also long-lived unstable isotopes (e.g. ^{238}U)
- Radioactive isotopes
  - are unstable nuclides, that decompose spontaneously until they achieve a stable configuration

The δ notation

- It is the most common notation used in isotope research. The δ value denotes the difference measurement made relative to a standard during the actual analysis.
- It is used because it amplifies small differences in the abundance of the rare isotope

$$\boxed{\delta = (R_{sample}/ R_{standard}) -1 * 1000}$$

Unit = ‰ (per mill, i.e. Latin for “per thousand”)

R = H/L, molar ratio of heavy-to-light isotopes of an element

Isotope Composition of International References Standards

Standard	Ratio, H/L	Value, H/L	%H	%L
St. Mean Ocean Water (SMOW)	$^{2}H/^{1}H$	0.00015576	0.015574	99.984426
	$^{17}O/^{16}O$	0.0003799	0.03790	99.76206
	$^{18}O/^{16}O$	0.0020052	0.20004	99.76206
PeeDee Belemnite (PDB)* and Vienna PeeDee Belemnite (VPDB)	$^{13}C/^{12}C$	0.011180	1.1056	98.8944
	$^{17}O/^{16}O$	0.0003859	0.0385	99.7553
	$^{18}O/^{16}O$	0.0020672	0.2062	99.7553
Air (AIR)	$^{15}N/^{14}N$	0.0036765	0.36630	99.63370
Canyon Diablo Troilite (CDT)** and Vienna Canyon Diablo Troilite (VCDT)	$^{33}S/^{32}S$	0.0078772	0.74865	95.03957
	$^{34}S/^{32}S$	0.0441626	4.19719	95.03957
	$^{36}S/^{32}S$	0.0001533	0.01459	95.03957

*Limestone from the PeeDee Formation in South Carolina (derived from the Cretaceous marine fossil *Belemnitella americana*)

**The Canyon Diablo triolite, is an irone sulfide meteorite, which impacted at Barringer Crater, in Arizona. It fragments were collected around the crater and nearby Canyon Diablo.

The Fractional notation

The fractional notations denote the proportion of the heavy isotope to the light isotope (R) or to the total (F).

R or F are the notation to use to calculate fractionation factors.

$R = H / L$ or $R = {}^{H}F / {}^{L}F$

${}^{H}F = H / (H+L)$

H = Heavy isotope
L = Light isotope

Atom% notation

Atom% or % Heavy isotope (^{H}AP) denotes the relative proportion, in percentage, of the heavy isotope in the sample. It is linearly correlated to δ values at lower ^{H}AP, but it is a better notation to use – gives exact values - at higher ^{H}AP, i.e. in isotope enrichment studies

$$^{H}AP = F * 100$$

$$^{H}AP = 100 * (\delta + 1000) / [(\delta +1000 + (1000/ R_{standard})]$$

How do we measure the stable C isotope composition of a sample?

Sample analysis

Isotope measurement:

- Isotope Ratio Mass Spectrometry (IRMS)
- Laser spectroscopy

Sample preparation:

- Type of sample, i.e. solid, liquid, gas
- Isotope (i.e.^{13}C, ^{15}N, ^{18}O, ^{2}H …)

IRMS

- Separation of charged atoms and molecules based on mass difference

- Element to be analyzed has to be in a stable and gaseous form (e.g. CO_2, N_2, O_2 ,H_2)

- Reaches high levels of precision, typically in the order of 0.1 to 1‰, depending on isotopes analyzed and on system (i.e. dual-inlet *vs* conflow)

- Essential components:
  - A **dual-inlet or a conflow system** for running sample and standard gas
  - A **ion source** (electron beam), where molecule are ionized by a hot filament
  - A **magnetic field,** where ions of different masses are resolved
  - **Faraday cups**, to collect and amplify the resolved ion beams
  - A **pumping system**, to maintain the system under vacuum
  - A **computer** for data acquisition and instrumental control

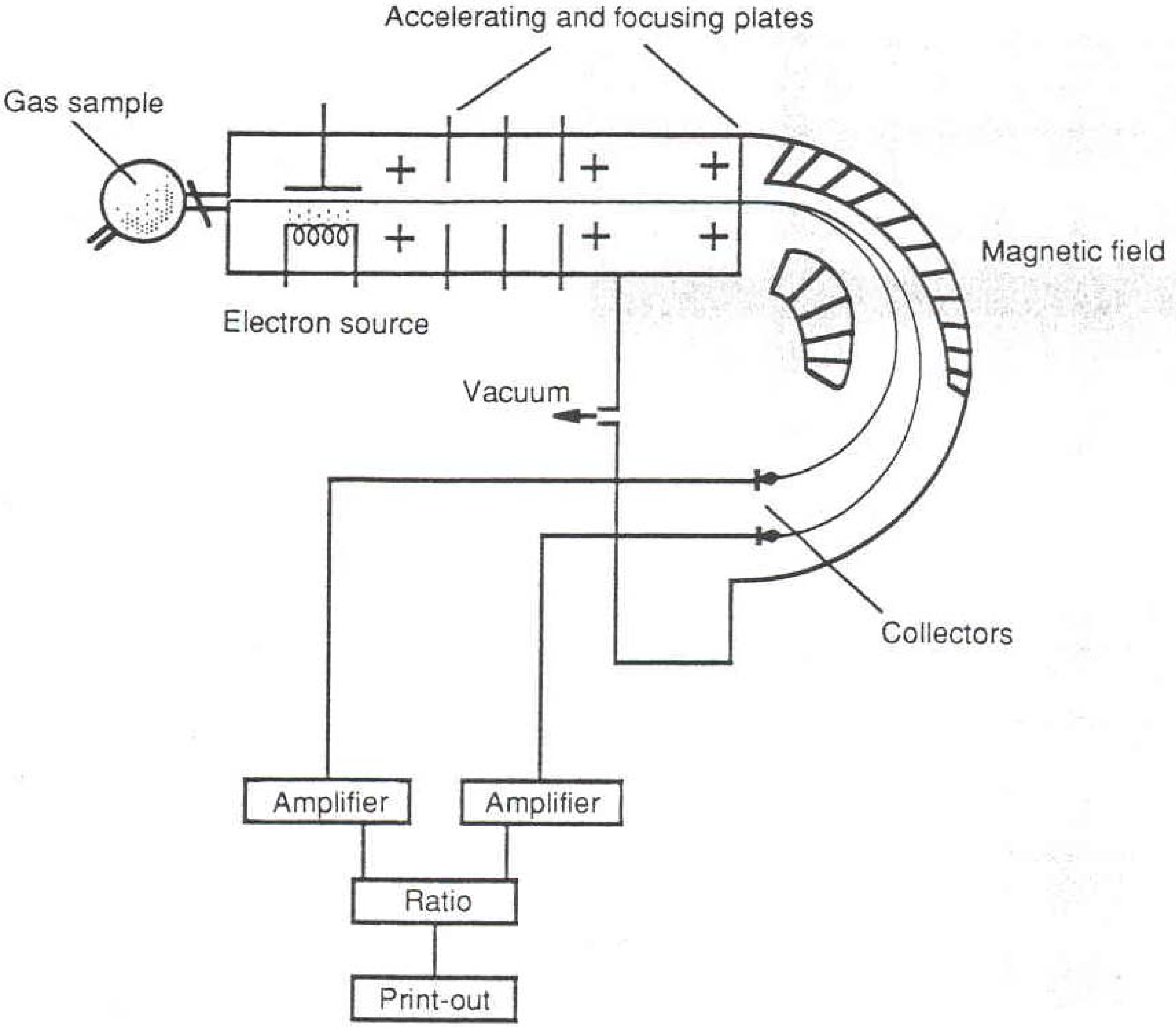


Figure 1 Schematic of a mass spectrometer. *(From Schimel, 1993)*

Sample preparation

Finalized to produce the element of interest from the form it is present in the sample to a form suitable for isotope analyses.

EXAMPLES:

Solid (i.e. plant, soils, bones, etc.) → CO_2, N_2, O_2, H_2

Liquid (i.e. water, extracts) → CO_2, N_2, O_2, H_2

Gas (i.e. atmosphere, GHG samples,..) → CO_2, N_2, O_2, H_2

Sample preparation must not cause isotopic fractionation!

Sample size

Is variable from lab to lab and depend on:

- ✓ The element concentration in the sample
- ✓ The volume of the IRMS inlet system
- ✓ The ionization efficiency of the ion source
- ✓ The degree of precision required

Always check with the lab manager about the optimum sample size for your samples!

Sample preparation for Dual inlet

Dual inlet system requires the preparation of pure gas samples off line.

Pure gas are generally stored in sealed glass tubes prior to analyses

A number of specific protocols are available for the preparation of gas samples depending on the sample type and isotope to be measured

Con-flow systems are replacing dual inlet in many Isotope laboratories, and this preparation are now seldom used

Sample preparation for Continuous flow method

Elemental analyzer (EA): commonly used for solid samples isotope analyses. Samples are combusted in a O_2 stream thus $\delta^{18}O$ can not be measured by this method.

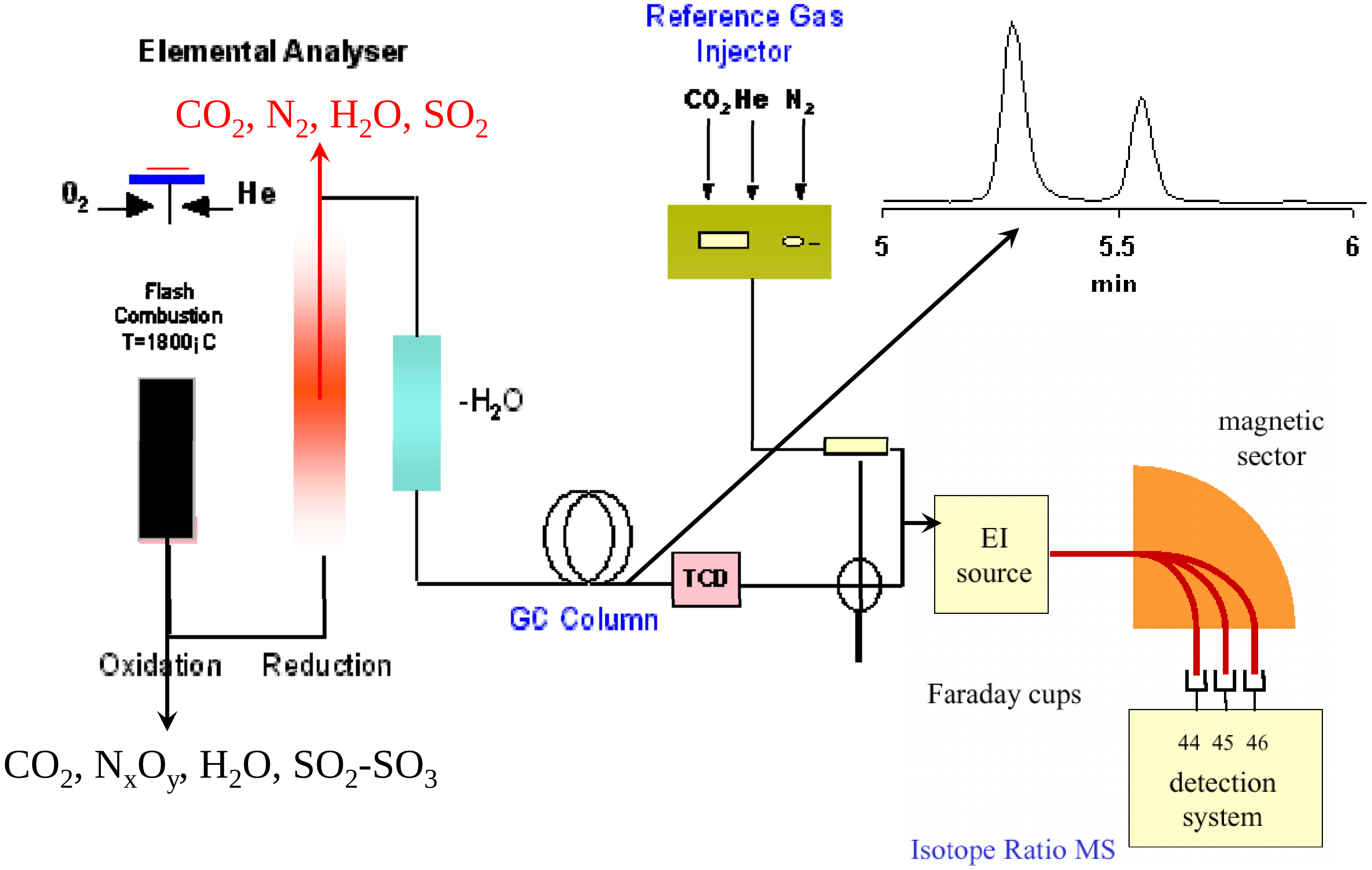
High Temperature C Reduction EA– (TC/EA): uses pyrolysis to decompose the samples, by high temperatures, into the gas molecule for IRMS analyses, thus allowing also for $\delta^{18}O$ of samples

Gas chromatographer (GC)/Pre-con systems: are columns used to concentrate gasses from lower concentrations typical of samples (e.g. ppm, ppb) to a pure form suitable for IRMS analyses

HPLC: allows the isotope analysis of active compounds with high polarity and high molar weight.

GC-TC: A capillary GC coupled with a high temperature pyrolysis is used to obtain compound specific isotope analyses of complex mixtures (i.e. PLFAs etc.)

Combustion - Isotope Ratio Mass Spectrometry



Drawbacks of IRMS

- Requires sample preparation
- Requires skillful operator
- It is expensive
- Instrumentation is generally bulky and confined to laboratory application _ *some field setting have been arranged*.

New laser apparatus are now available for continuous analyses of gas samples

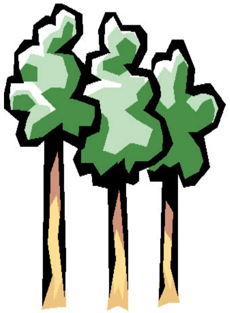
Natural abundance of stable C isotopes

δ^{13}C of major components of terrestrial ecosystems

Atmospheric CO_2
-8‰ / -10 ‰

Anthropogenic CO_2
-27‰

C3 plants
-24‰ / -30 ‰



C4 plants
-16‰ / -12 ‰

CAM plants
-12‰ / -28 ‰

Litter	-24‰ / -28‰	-13‰	-24 ‰
Soil CO_2	-19‰ / -23‰	-8 ‰	-19 ‰
Pedogenic Rocks:	-12‰	+2 ‰	

Litogenic Carbonates
- 0‰

Coal
-25‰

Natural gases
-40‰

Petroleum
-30‰

Why is there variation in the stable C isotope composition of different ecosystem components?

Isotope fractionation

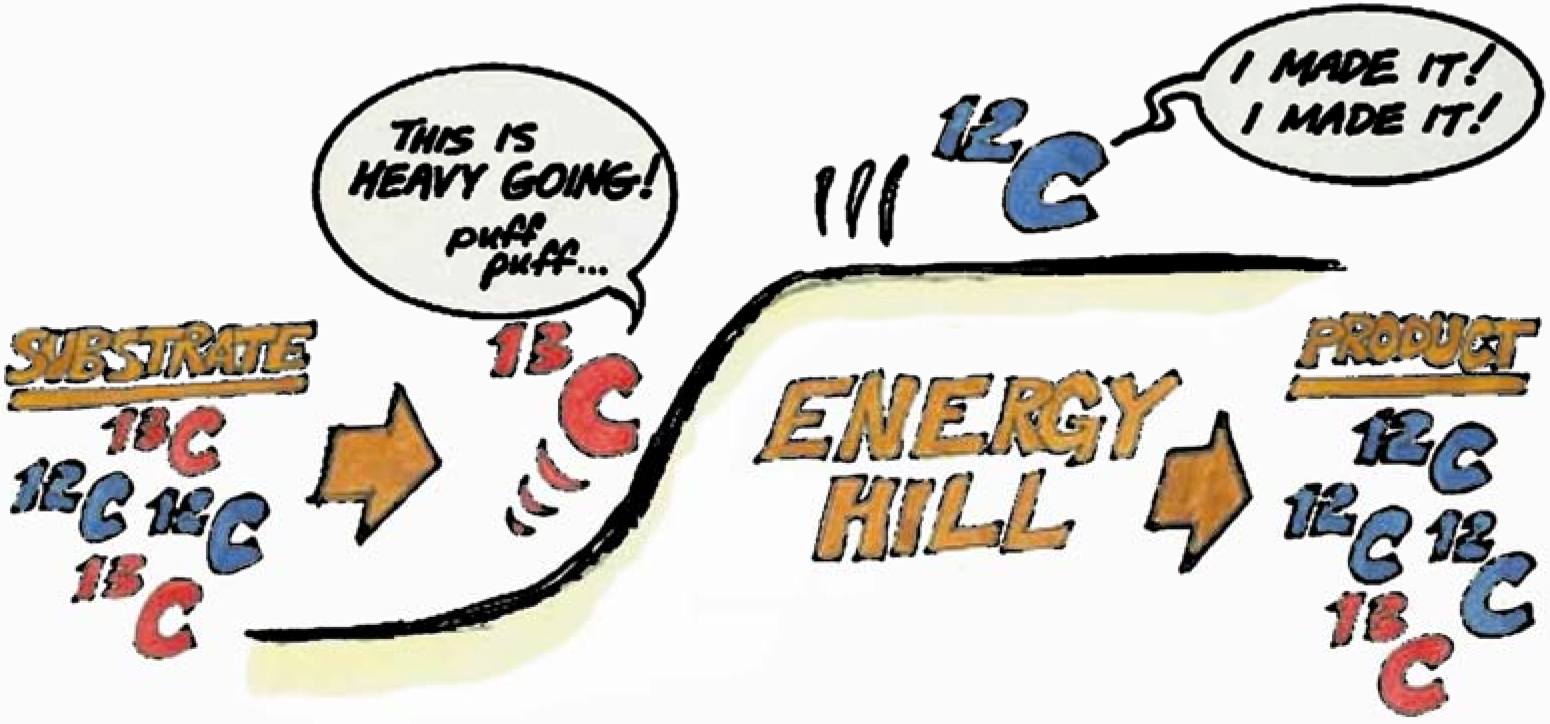
Chemical properties of isotopes

different isotopes of a given element all have the same number of protons and electrons and the same electronic structure, and because the chemical behavior of an atom is largely determined by its electronic structure

different isotopes exhibit nearly identical chemical behavior

However …..

SOMETIMES THE EXTRA NEUTRON MAKES A DIFFERENCE. IT'S HARDER TO PUSH THE HEAVY MOLECULES UP AN ENERGY HILL…



… SO THAT PRODUCTS HAVE MORE OF THE LIGHT ISOTOPE AND LESS OF THE HEAVY ISOTOPE.

Fry, 2006

Fractionation processes

Fractionation may occur in:

- Chemical reactions: **Kinetic fractionation**
- Thermodynamic equilibrium: **Equilibrium fractionation**
- Physical processes: **Physical fractionation**

Chemical fractionation

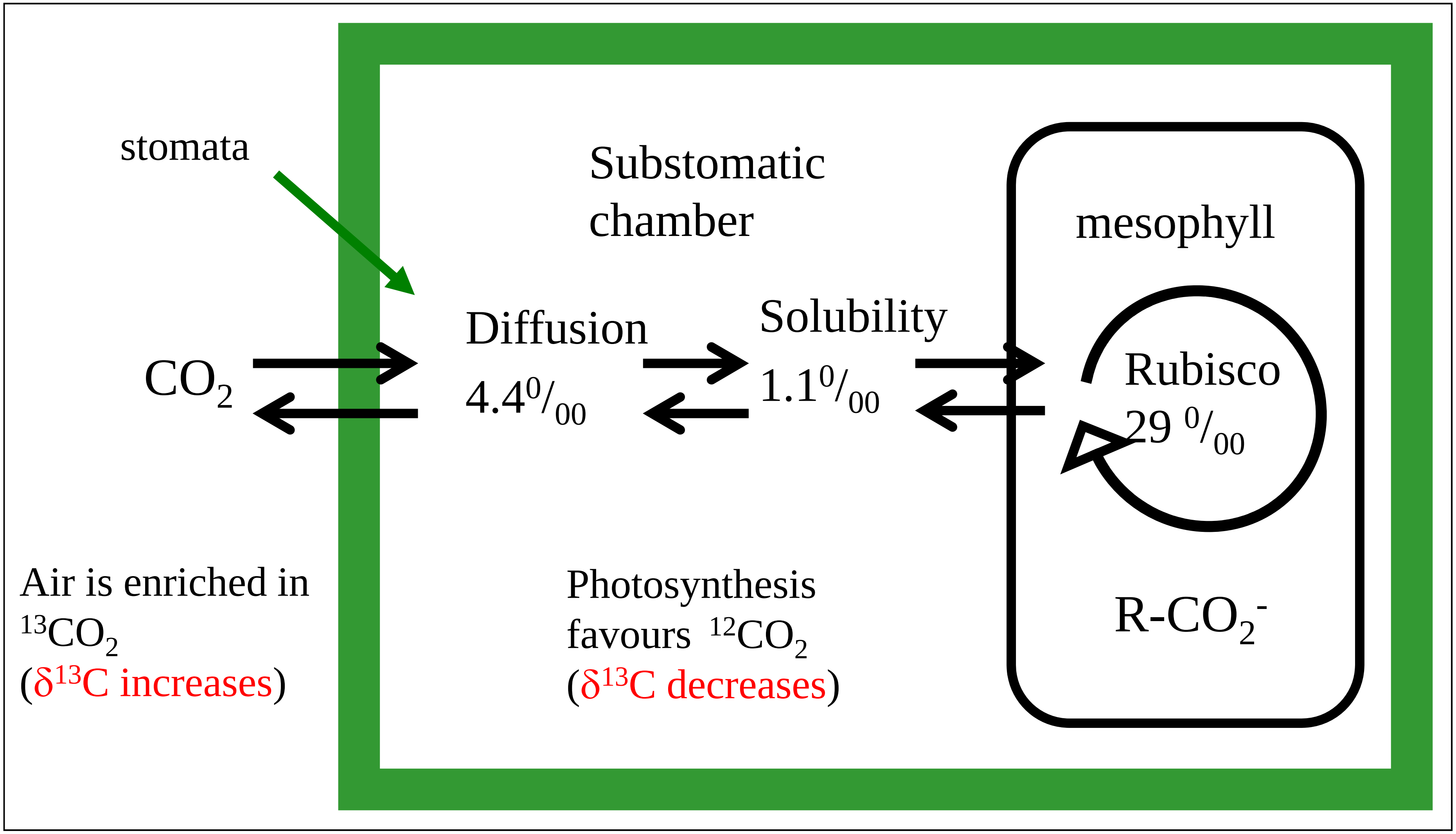
Atoms with extra neutrons (i.e. heavy isotopes) make stronger bonds, that require more activation energy to form but also more energy to brake.

Isotopes affect the *vibrational* component of bonds.

For this reason chemical or kinetic fractionation occurs at the atomic level when different isotopes are involved in the reaction.

C isotope fractionation during photosynthesis in C3 plants

Farquar et al., 1989



stomata

Substomatic chamber

mesophyll

CO_2

Diffusion

$4.4\,{}^{0}/_{00}$

Solubility

$1.1\,{}^{0}/_{00}$

Rubisco

$29\ {}^{0}/_{00}$

Air is enriched in $^{13}CO_2$ ($\delta^{13}C$ increases)

Photosynthesis favours $^{12}CO_2$ ($\delta^{13}C$ decreases)

$R\text{-}CO_2^-$

C isotope fractionation during photosynthesis in C3 plants

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fractionation= $[a + (b - a)\, p_i/p_a – d]$

a= diffusion fractionation, 4.4 ‰*

b= carboxylation + solubility, 30 ‰

d= contribution by a variety of minor factors – often negligible

p_i= internal CO_2 partial pressure

p_a= atmospheric CO_2 partial pressure

*Note that in this case positive value indicate that the product is depleted!

Farquar et al., 1989

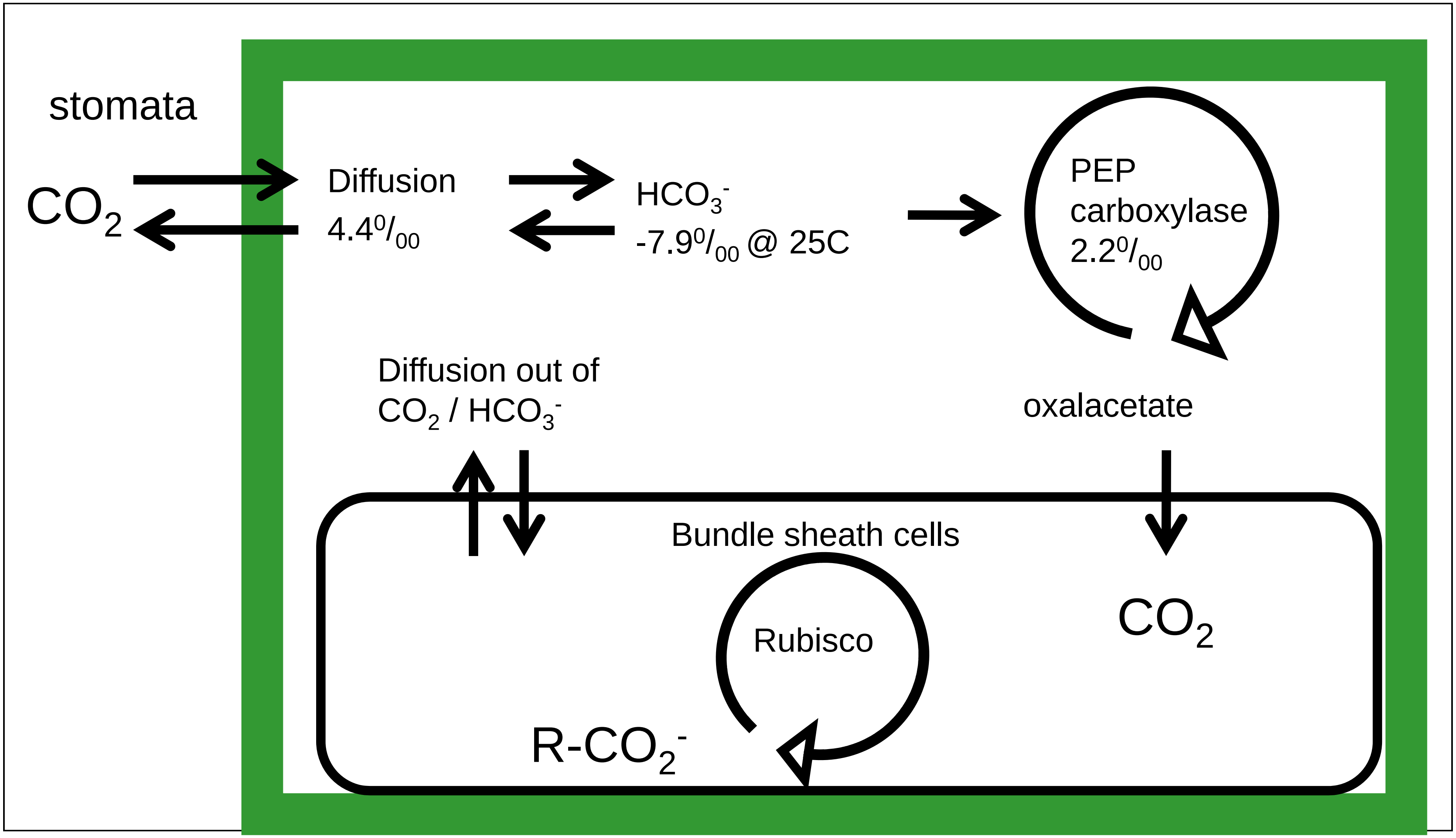
C isotope fractionation during photosynthesis in C3 plants

Theoretically, $\delta^{13}C$ in C3 plants could range from a value of -12 ‰, when diffusion is the limiting step, stomata are almost closed and all the CO_2 is used for carboxylation, to a value of -38‰, when stomata are completely open and diffusion does not express.

In reality the $\delta^{13}C$ is in between those values, and it is an index of C3 plant water use efficiency!

Farquhar et al., 1989

C isotope fractionation during photosynthesis in C4 plants



stomata

CO_2

Diffusion

$4.4^{0}/_{00}$

HCO_3^-

$-7.9^{0}/_{00}$ @ 25C

PEP carboxylase

$2.2^{0}/_{00}$

Diffusion out of

CO_2 / HCO_3^-

oxalacetate

Bundle sheath cells

Rubisco

CO_2

$R\text{-}CO_2^-$

C isotope fractionation during photosynthesis in C4 plants

$$\delta^{13}C_{plant}= \delta^{13}C_{atm} -a - (c + b\phi - a)\ p_i/p_a$$

a= diffusion fractionation, 4.4 ‰*

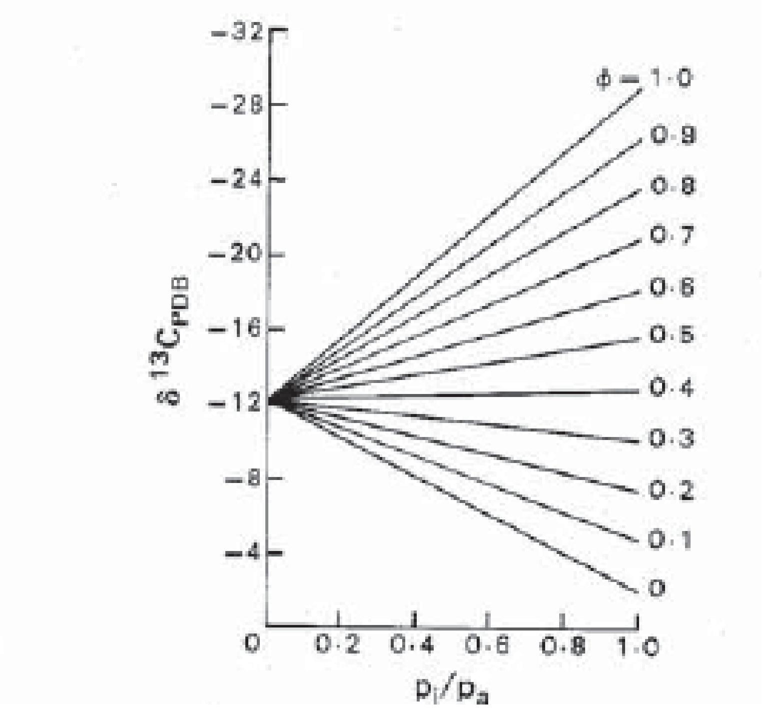
b= carboxylation, 29 ‰

c= carbonic anidrase + PEP (-7.9 + 2.2 ‰)

p_i= internal CO_2 partial pressure

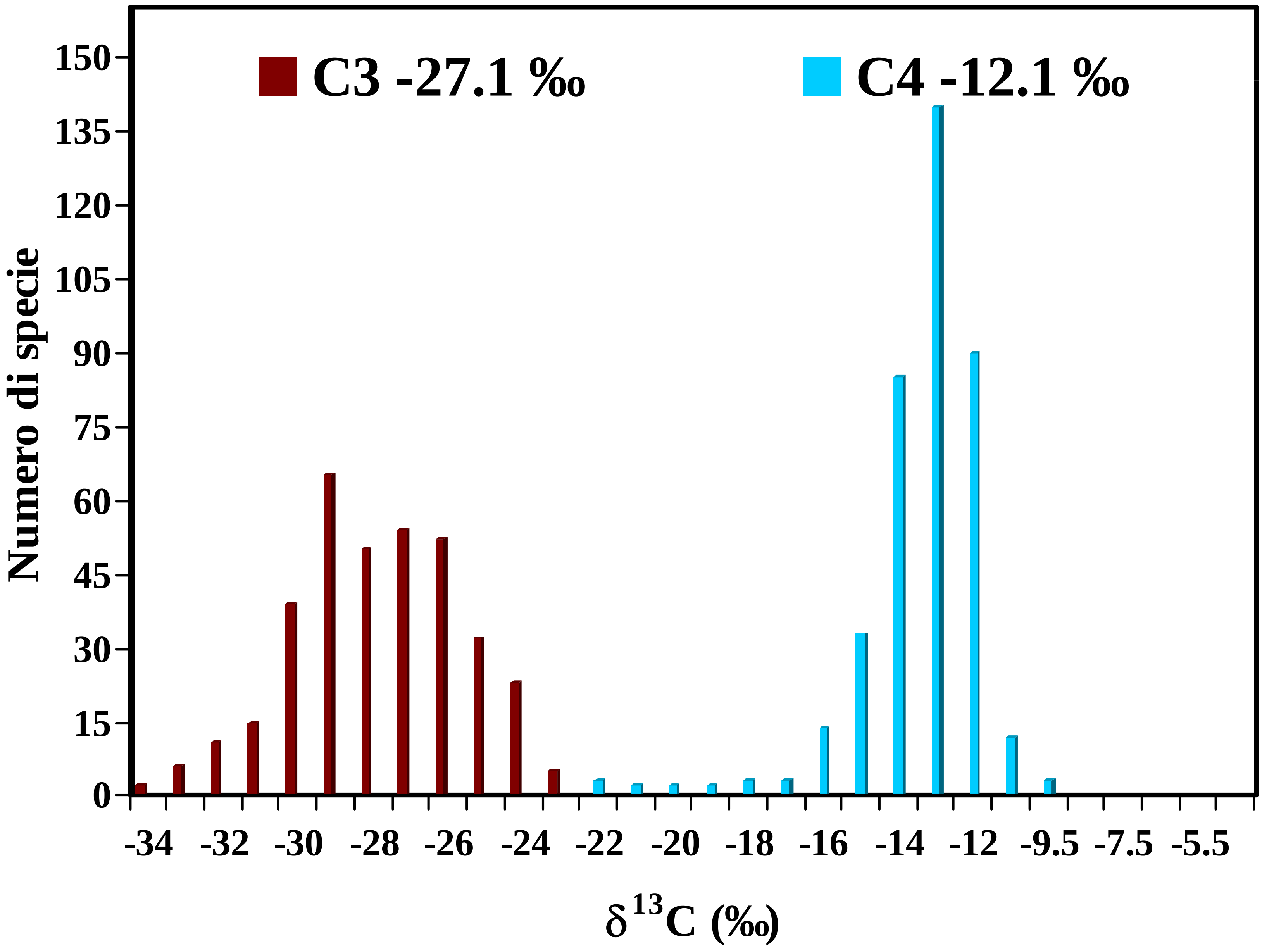
p_a= atmospheric CO_2 partial pressure

ϕ = leakage factor



*Note that in this case positive value indicate that the product is depleted!

Farquar et al., 1983


C3 -27.1 ‰
C4 -12.1 ‰
Numero di specie
150
135
120
105
90
75
60
45
30
15
0
-34
-32
-30
-28
-26
-24
-22
-20
-18
-16
-14
-12
-9.5
-7.5
-5.5
δ^{13}C (‰)

$\delta^{13}C$ of soil organic matter (SOM) is a direct function of the vegetation from which it was formed

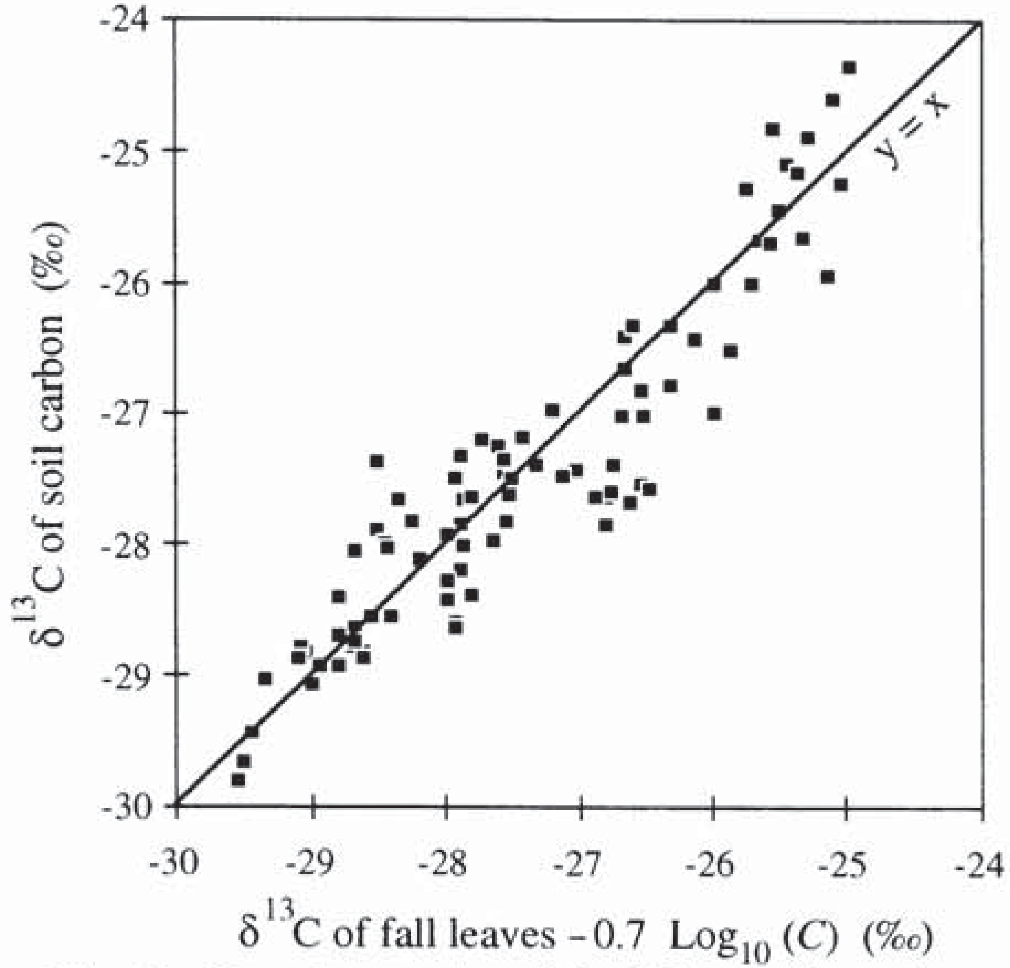


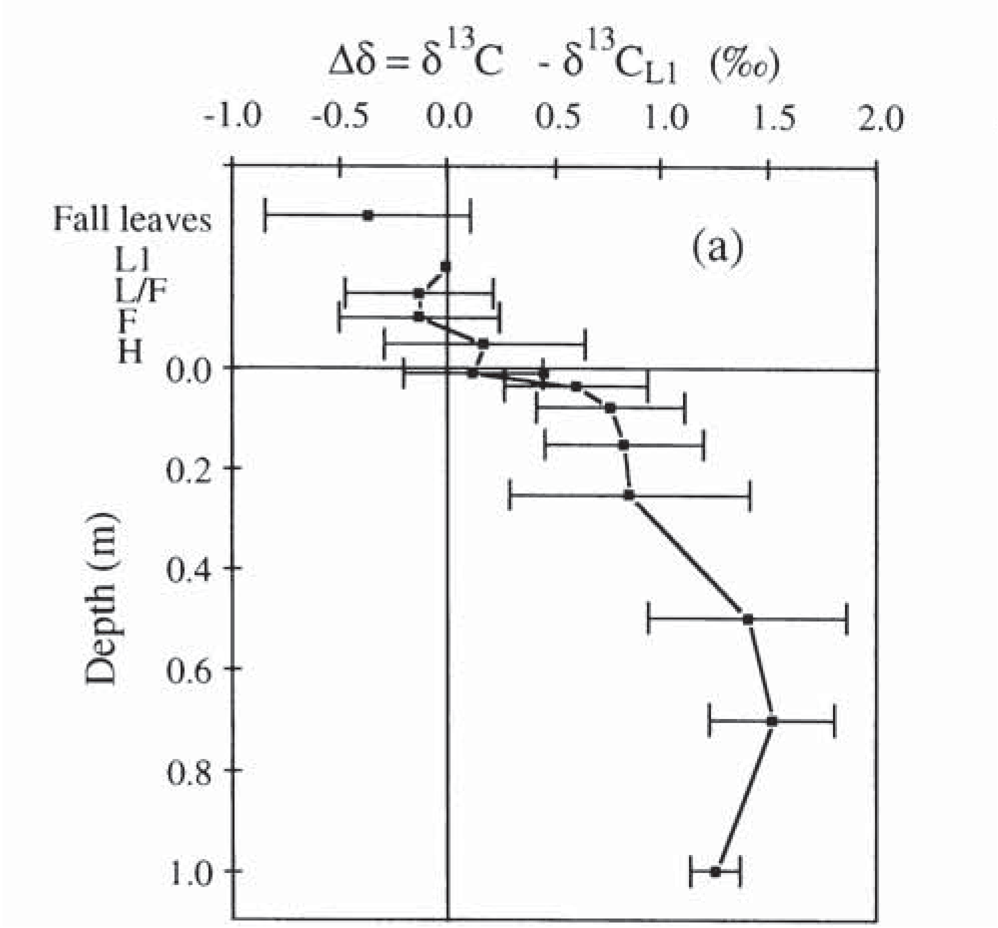
FIG. 4. Overall regression of the $\delta^{13}C$ of soil samples on the $\delta^{13}C$ of leaves falling on the soil corrected for soil sample carbon concentration (C in mass per unit mass) (see discussion of Eq. 1).

Balesdent et al., 1993

δ^{13}C enrichment along the soil profile with reference to the litter

This is a very conservative observation across studies.

In this particular study the same trend was observed, irrespective of soil type, physico-chemical characteristics, etc.

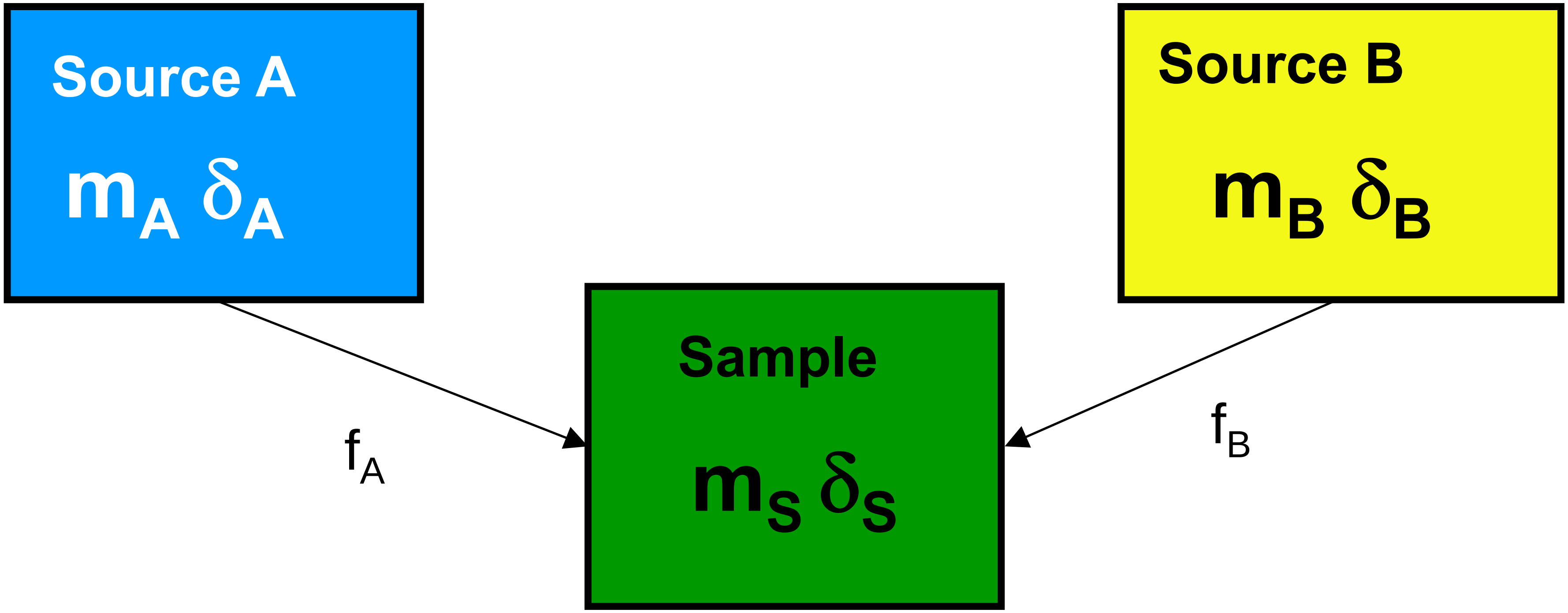


Balesdent et al., 1993

How can we make use of variations in the **natural abundance** of stable C isotope for studies of soil organic carbon dynamics?

Applying the mass balance approach

Mass balance



Source A

$\mathbf{m_A\ \delta_A}$

Source B

$\mathbf{m_B\ \delta_B}$

Sample

$\mathbf{m_S\ \delta_S}$

f_A

f_B

$\delta_S m_S = \delta_A * m_A + \delta_B * m_B$

$f_A = m_A / m_S$

$m_S = m_A + m_B$

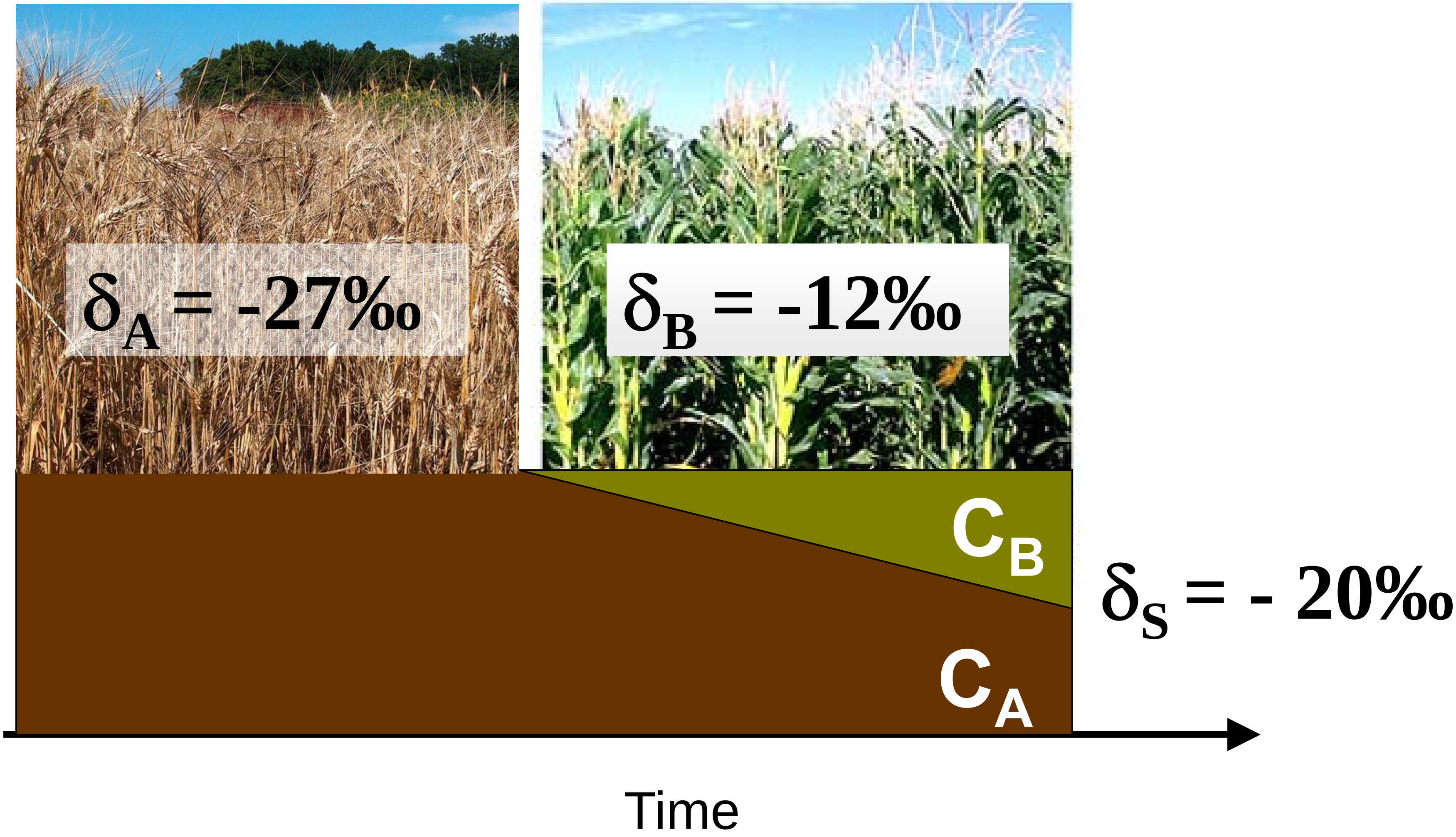
$f_B = m_B / m_S = 1 - f_A$

$$\boldsymbol{\delta_S = (m_A\delta_A + m_B\delta_B)/(m_A + m_B)}$$

$$\boldsymbol{f_A = (\delta_S - \delta_B)/(\delta_A - \delta_B)} \qquad \boldsymbol{m_A = m_S * f_A}$$

Appling the mixing model …

we can quantify the relative contribution of each vegetation type to the soil organic carbon pool

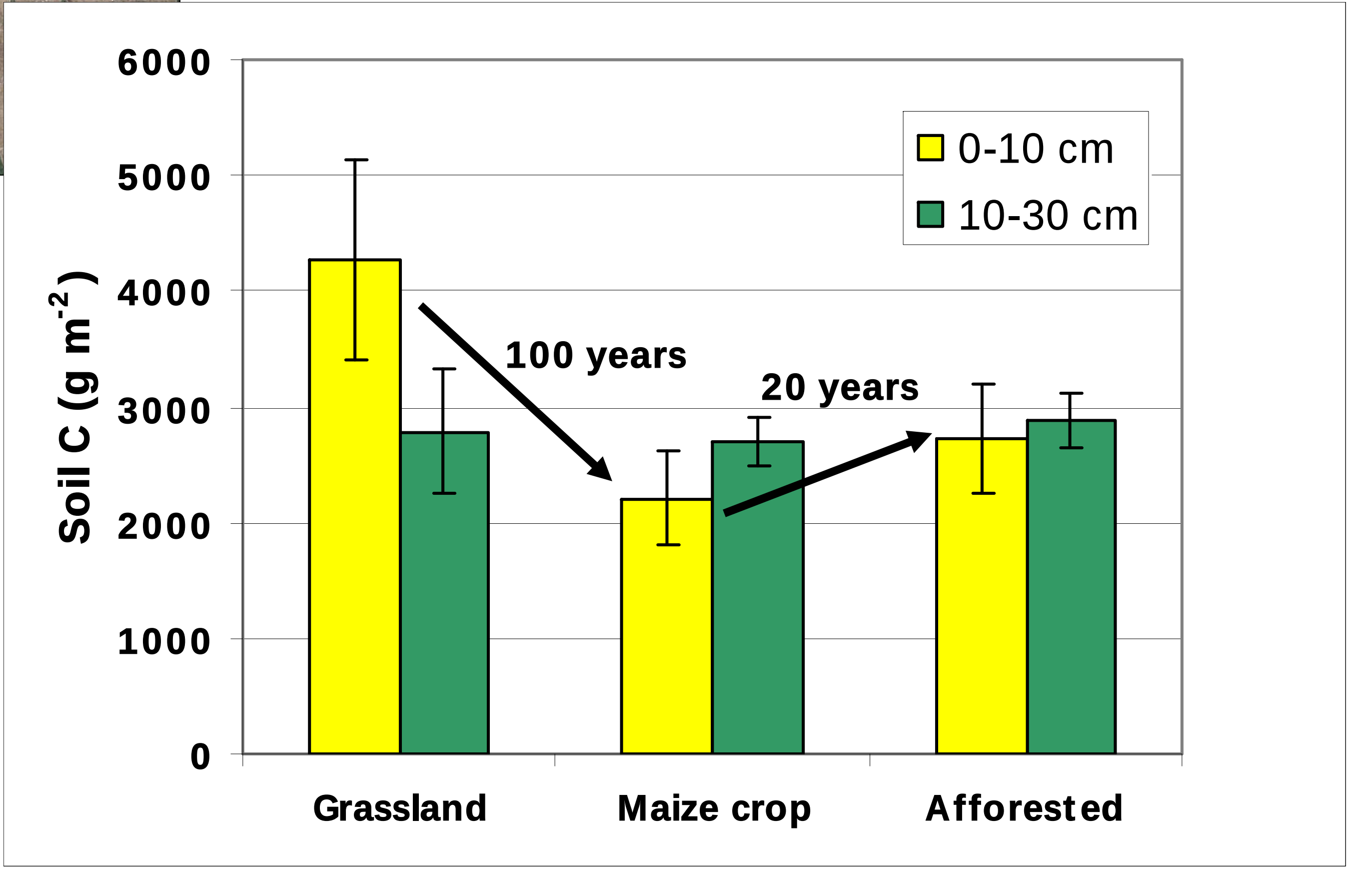


$f_B = ((-20) – (-27))/((-12) – (-27)) = 7/15 = 0.47$

This approach can be applied to the bulk soils, to specific SOM fractions, as well as to partition soil respiration

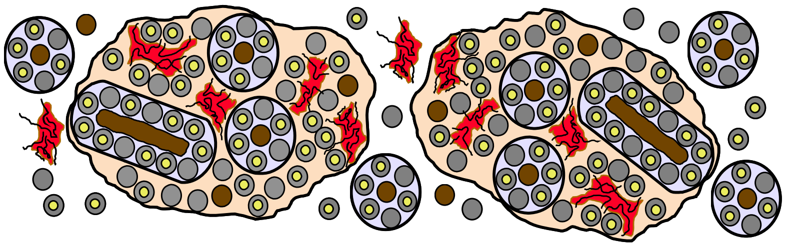
Effects of land use change on soil C:

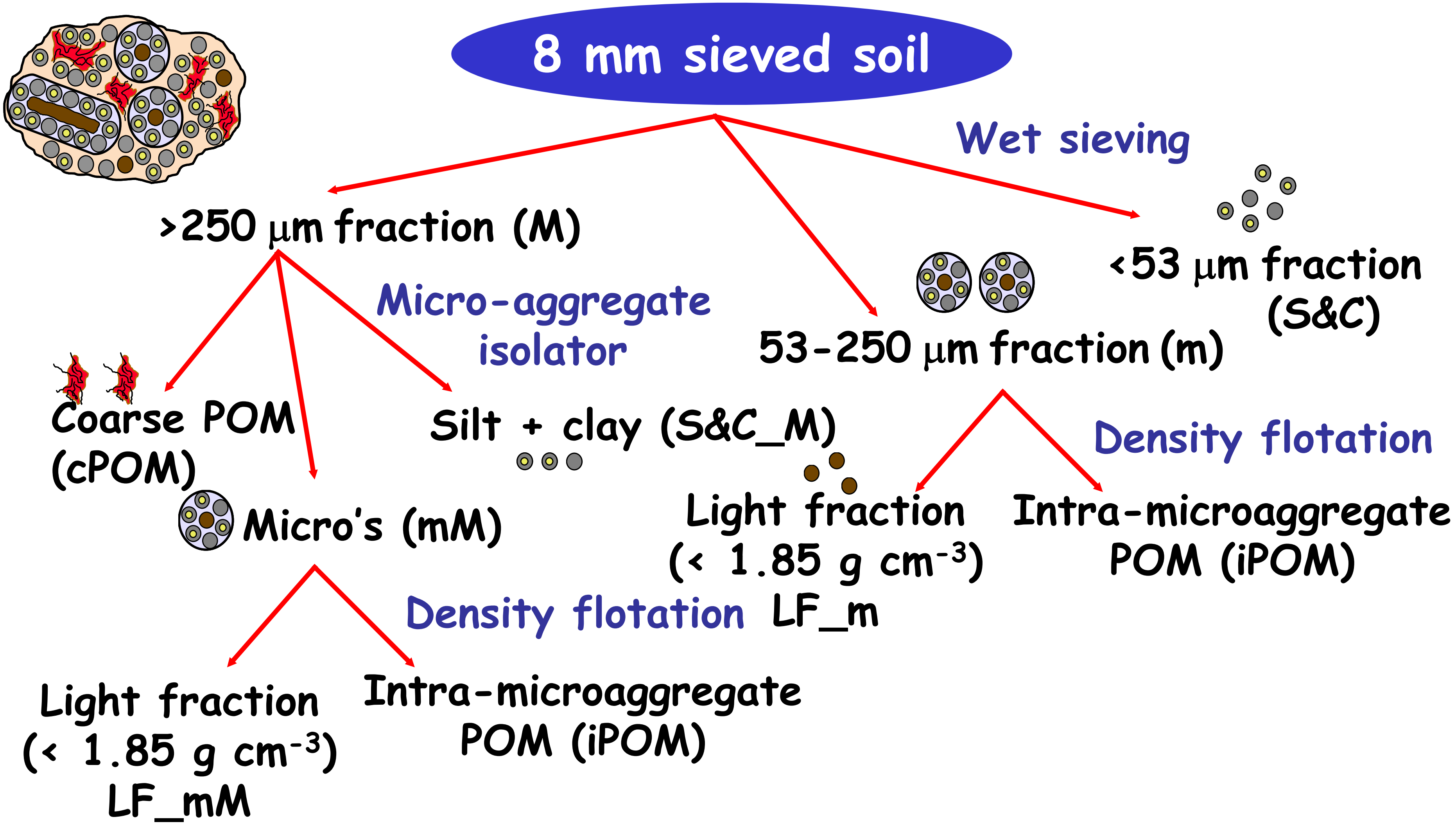
a case study in Northern East Italy



Del Galdo et al., 2003

Fractionation of SOM by size and density





8 mm sieved soil

Wet sieving

>250 μm fraction (M)

53-250 μm fraction (m)

<53 μm fraction (S&C)

Micro-aggregate isolator

Coarse POM (cPOM)

Silt + clay (S&C_M)

Micro's (mM)

Density flotation

Light fraction (< 1.85 g cm^{-3}) LF_mM

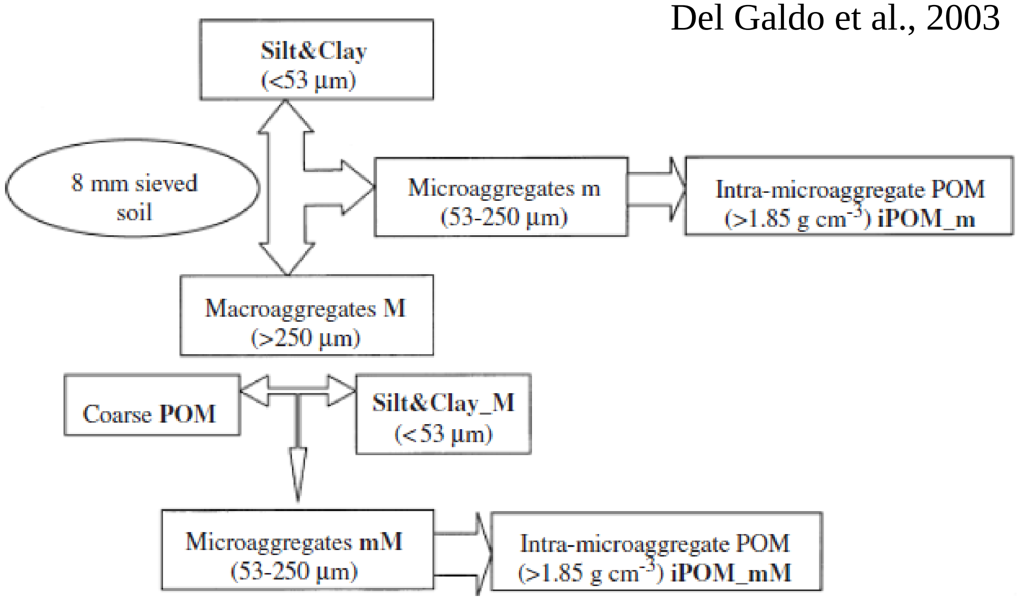
Intra-microaggregate POM (iPOM)

Density flotation

Light fraction (< 1.85 g cm^{-3}) LF_m

Intra-microaggregate POM (iPOM)

Del Galdo et al., 2003


8 mm sieved soil

Silt&Clay (<53 µm)

Microaggregates m (53-250 µm)

Intra-microaggregate POM (>1.85 g cm^{-3}) **iPOM_m**

Macroaggregates M (>250 µm)

Coarse POM

Silt&Clay_M (<53 µm)

Microaggregates mM (53-250 µm)

Intra-microaggregate POM (>1.85 g cm^{-3}) **iPOM_mM**

- ➢Bulk soil was fractionated by size and density
- ➢ Individual fractions were analyzed for C% and d^{13}C.
- ➢Two end-members mixing model was used to partition soil stocks in each fraction between the new and old C

$$f_{\mathrm{new}} = (\delta_{\mathrm{new}} - \delta_{\mathrm{old}})/\delta_{\mathrm{veg}} - \delta_{\mathrm{old}}$$

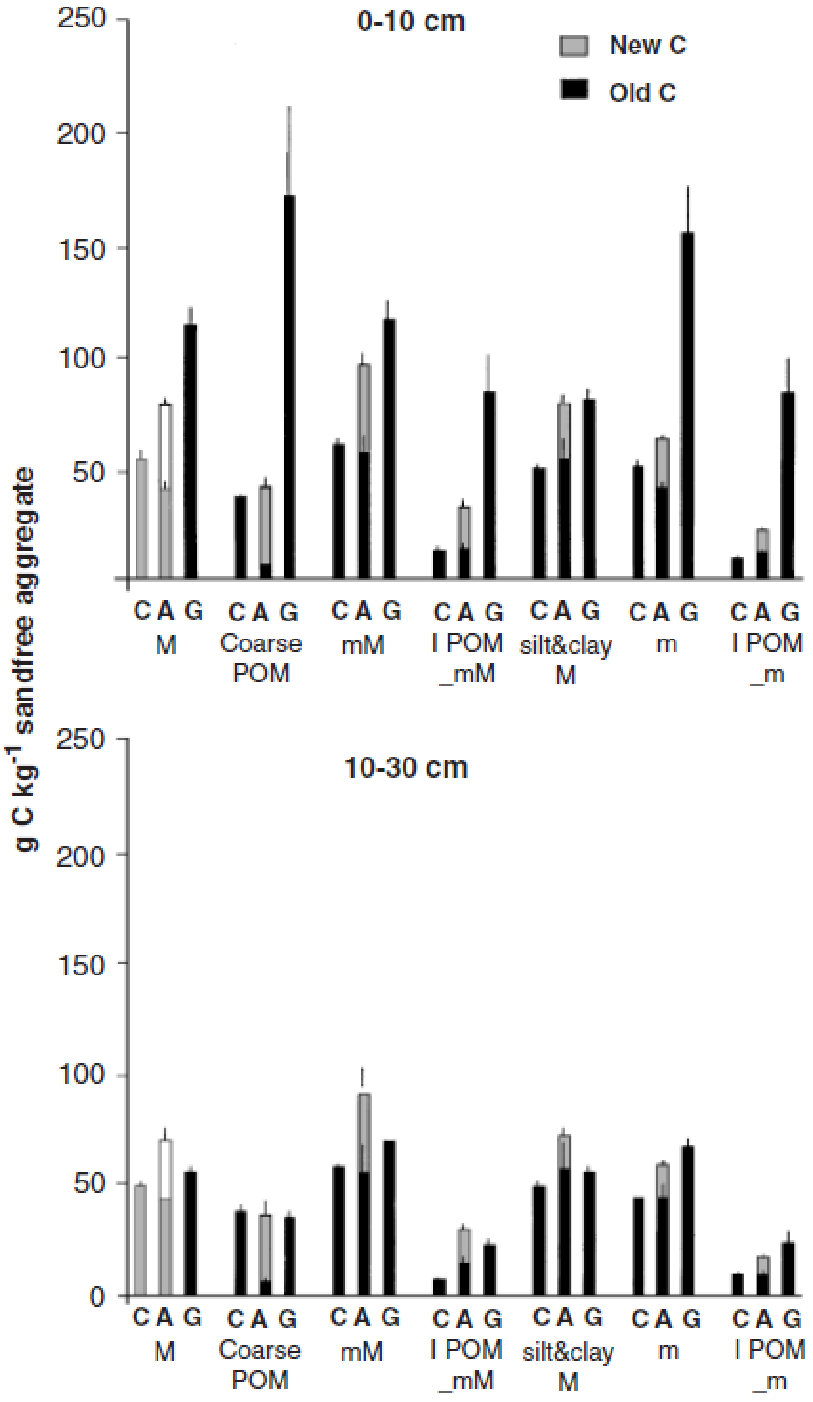
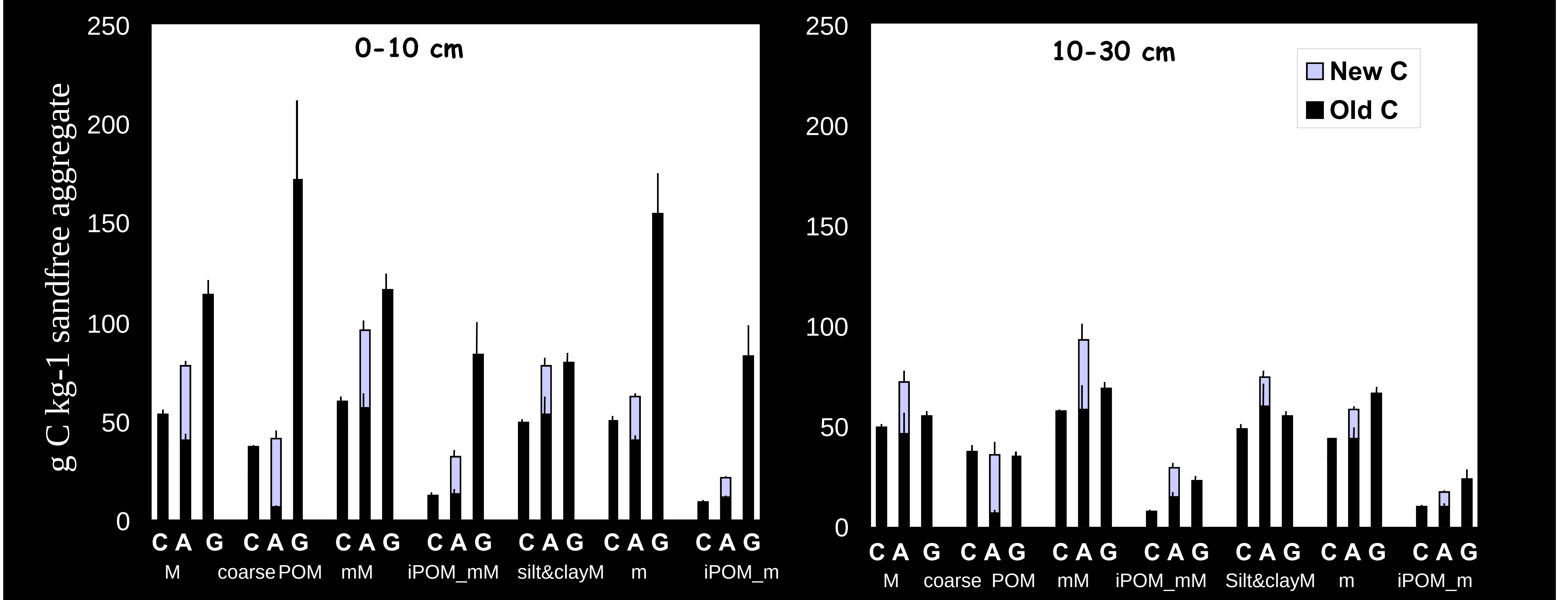


Fig. 2 Concentrations of C in the SOM fractions, at two soil depths, for the cropped (C), the afforested (A) and the grassland (G) systems. For afforested soils, the old (i.e. previous to land-use change) and the new C are indicated. Acronyms as in Fig. 1.

Partition of soil C into:

“new” - C derived from vegetation
“old” – native SOC

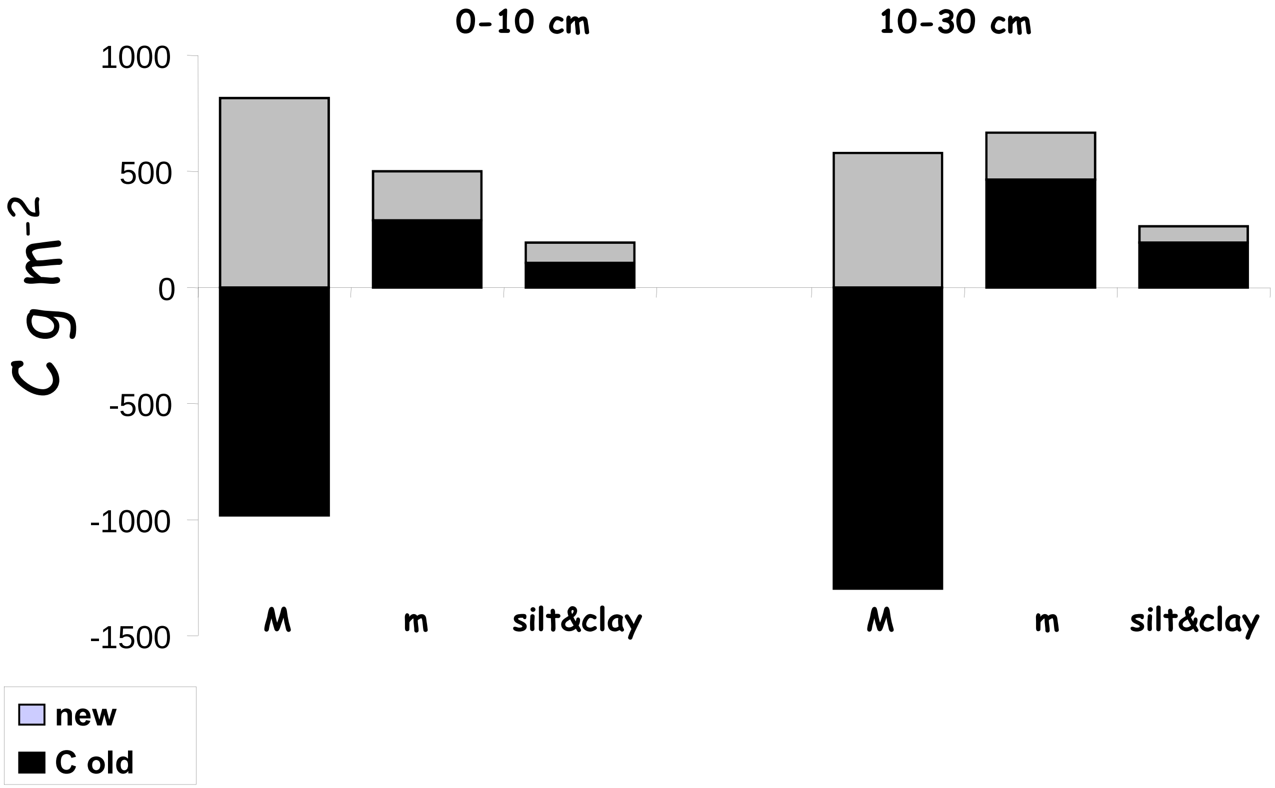


C= Crop
A=Afforested
G=Grassland

Del Galdo et al., *GCB*, 2003

Identifying SOC dynamics

Changes of C pools in different aggregate fractions in the afforested soil with reference to the crop soil. Isotopes reveled the partitioning between old and new C and allowed tracing C fluxes from one pool into an other.



Del Galdo et al., 2003

Assuming steady state, turnover times of SOC fractions can be calculated

Decomposition rate (y^{-1}) calculated for the old carbon of all measured physical fractions of afforested soil assuming an eponential decay {$k=-\ln(C_{old}/C_{total})/t$}. Default values decomposition rates of Roth C model are reported for comparison. Standard errors in parenthesis.n.d.= not detectable.

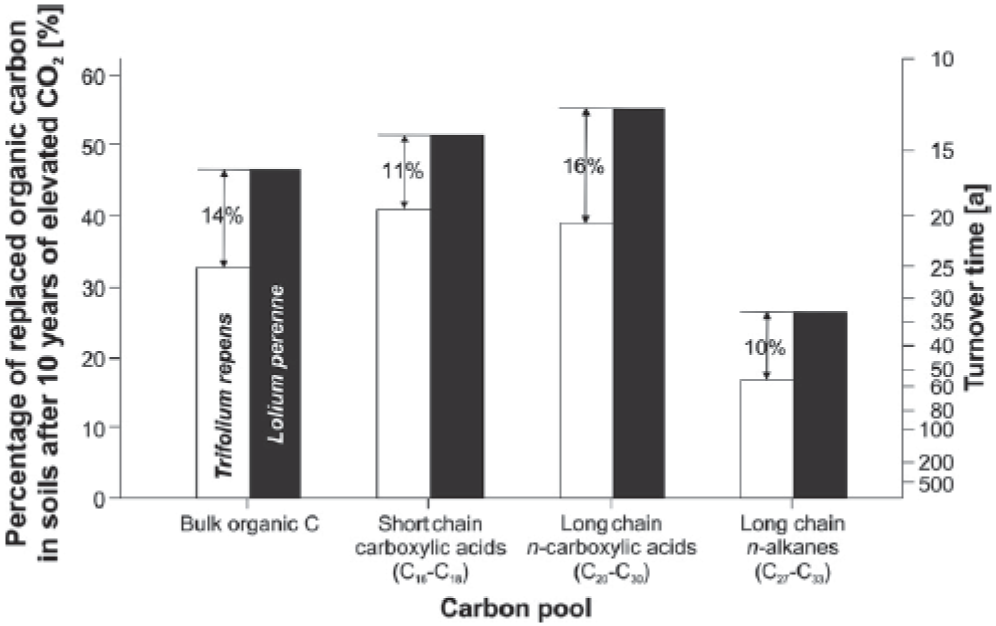
Measured decomposition rate factors (y^{-1})

M > 250 μm					**m** 53-250 μm		**Silt&clay** < 53 μm
Total Fraction	Coarse POM	mM	IPOM mM	silt&clayM	Total Fraction	IPOM m	Total Fraction
0.039 (>0.3)	0.108 (>0.3)	0.042 (>0.3)	0.024 (>0.3)	0.008 (>0.3)	n.d.	n.d.	n.d.

RothC decomposition rate factors (y^{-1})

Decomposable Plant Material DPM	Resistant Plant Material RPM	Humified Organic Matter HUM	Microbial Biomass BIO	Inert Organic Matter IOM
10	0.3	0.02	0.66	0.00

Quantifying microbial and other SOM pools turnover rates by stable isotope analyses



Method requirements:

- ➢ A change in the isotopic composition of C input at a known time

Method assumptions:

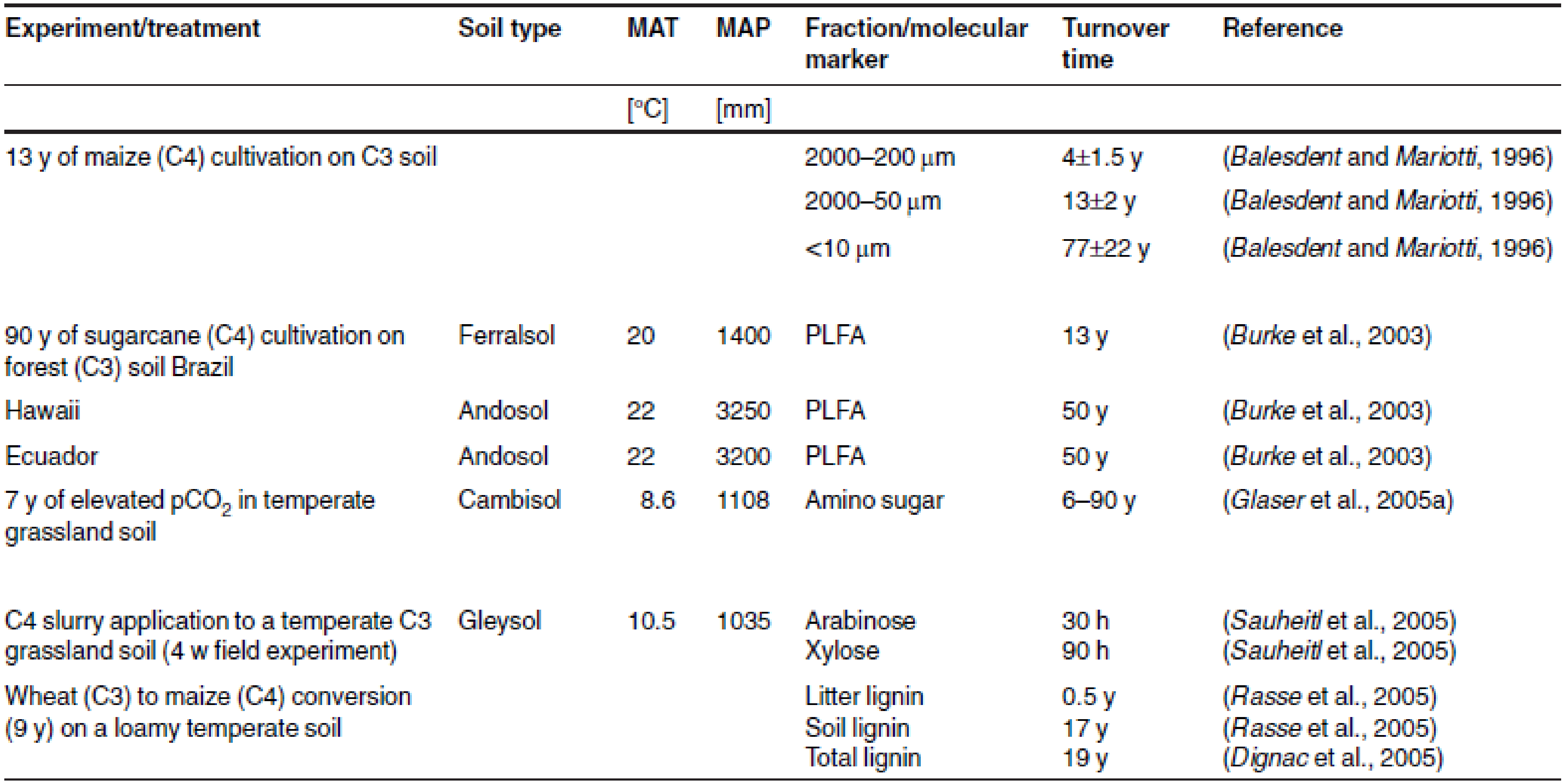
- ➢ System is a steady-state
- ➢ The isotopic composition of the control SOM is equal to the isotopic composition of SOM prior to change

Decay rate, $k = \ln (F_{old\ t} / F_{old\ t0}) / t-t0$

Turnover time = $1/k$

Wiesenberg et al., 2008

Quantifying microbial and other SOM pools turnover rates by stable isotope analyses



Experiment/treatment	Soil type	MAT	MAP	Fraction/molecular marker	Turnover time	Reference
		[°C]	[mm]			
13 y of maize (C4) cultivation on C3 soil				2000–200 µm	4±1.5 y	(*Balesdent* and *Mariotti*, 1996)
				2000–50 µm	13±2 y	(*Balesdent* and *Mariotti*, 1996)
				<10 µm	77±22 y	(*Balesdent* and *Mariotti*, 1996)
90 y of sugarcane (C4) cultivation on forest (C3) soil Brazil	Ferralsol	20	1400	PLFA	13 y	(*Burke* et al., 2003)
Hawaii	Andosol	22	3250	PLFA	50 y	(*Burke* et al., 2003)
Ecuador	Andosol	22	3200	PLFA	50 y	(*Burke* et al., 2003)
7 y of elevated pCO_2 in temperate grassland soil	Cambisol	8.6	1108	Amino sugar	6–90 y	(*Glaser* et al., 2005a)
C4 slurry application to a temperate C3 grassland soil (4 w field experiment)	Gleysol	10.5	1035	Arabinose Xylose	30 h 90 h	(*Sauheitl* et al., 2005) (*Sauheitl* et al., 2005)
Wheat (C3) to maize (C4) conversion (9 y) on a loamy temperate soil				Litter lignin Soil lignin Total lignin	0.5 y 17 y 19 y	(*Rasse* et al., 2005) (*Rasse* et al., 2005) (*Dignac* et al., 2005)

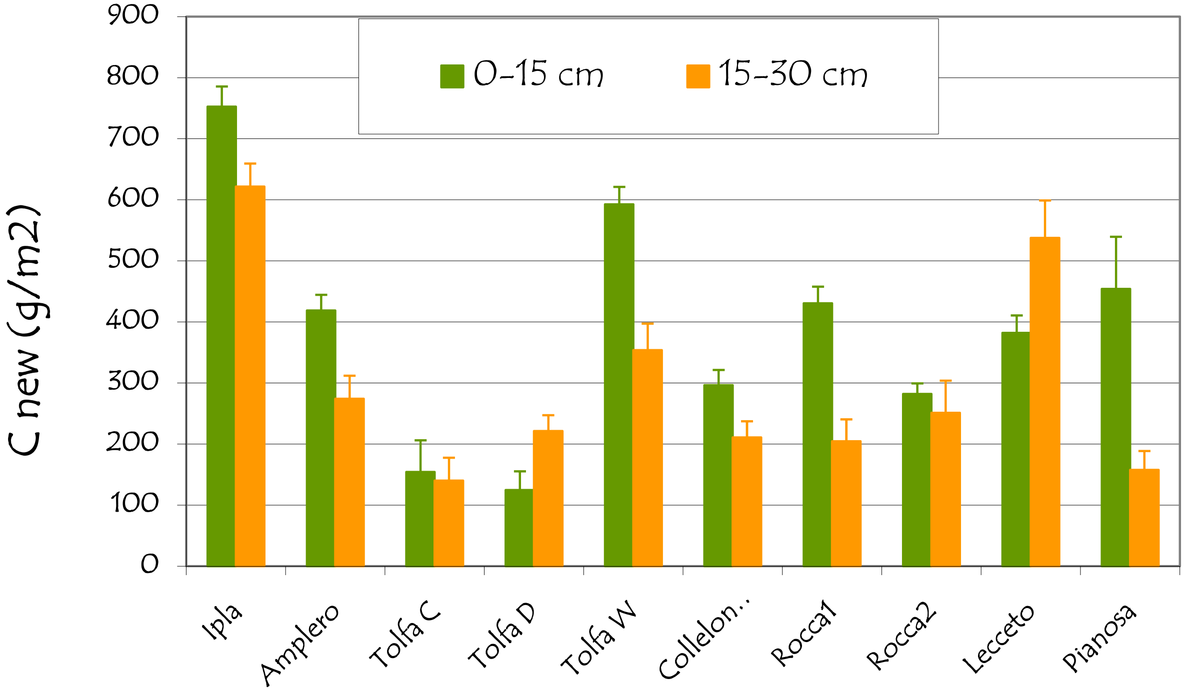
Glaser, 2005

Quantification of net C input in terrestrial ecosystems: a case study on systems of the Italian peninsula, under different land-uses

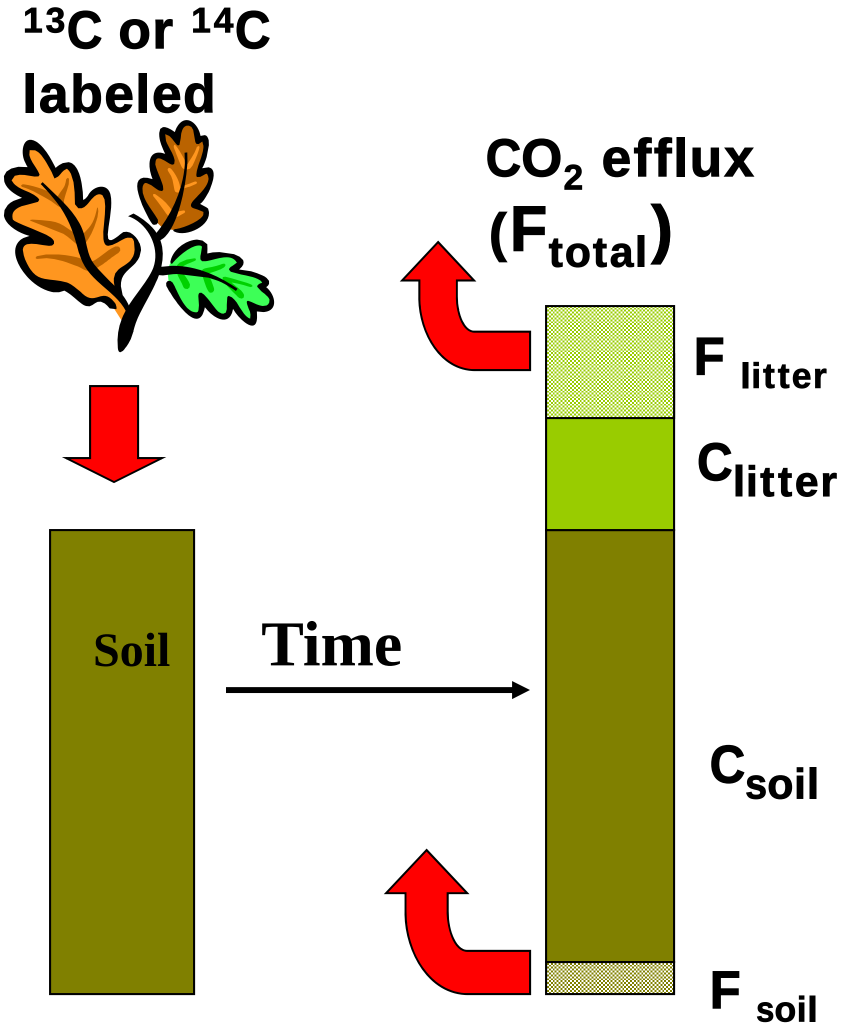
C4 soil cores in C3 systems: the method utilizes the different isotopic signal of a soil developed under C4 vegetation ($\delta^{13}C \approx -17‰$) and the existing C3 vegetation ($\delta^{13}C \approx -26‰$)

C4 soil cores (30 cm h, 4 cm Ø) are inserted in the soil of a C3 site The mesh of the net rounding the soil (2 mm) only allows the "ingrowth" of fine roots.

At all systems C new was less that 1 % of the total C and could have never been detected using conventional methods



Partitioning litter *vs* soil respiration: RATIONAL



^{13}C or ^{14}C labeled

Soil

Time

CO_2 efflux (F_{total})

F_{litter}

C_{litter}

C_{soil}

F_{soil}

$$f_{litter} = \delta_{total} - \delta_{soil} / \delta_{litter} - \delta_{soil}$$

$$F_{litter} = f_{litter} * F_{total}$$

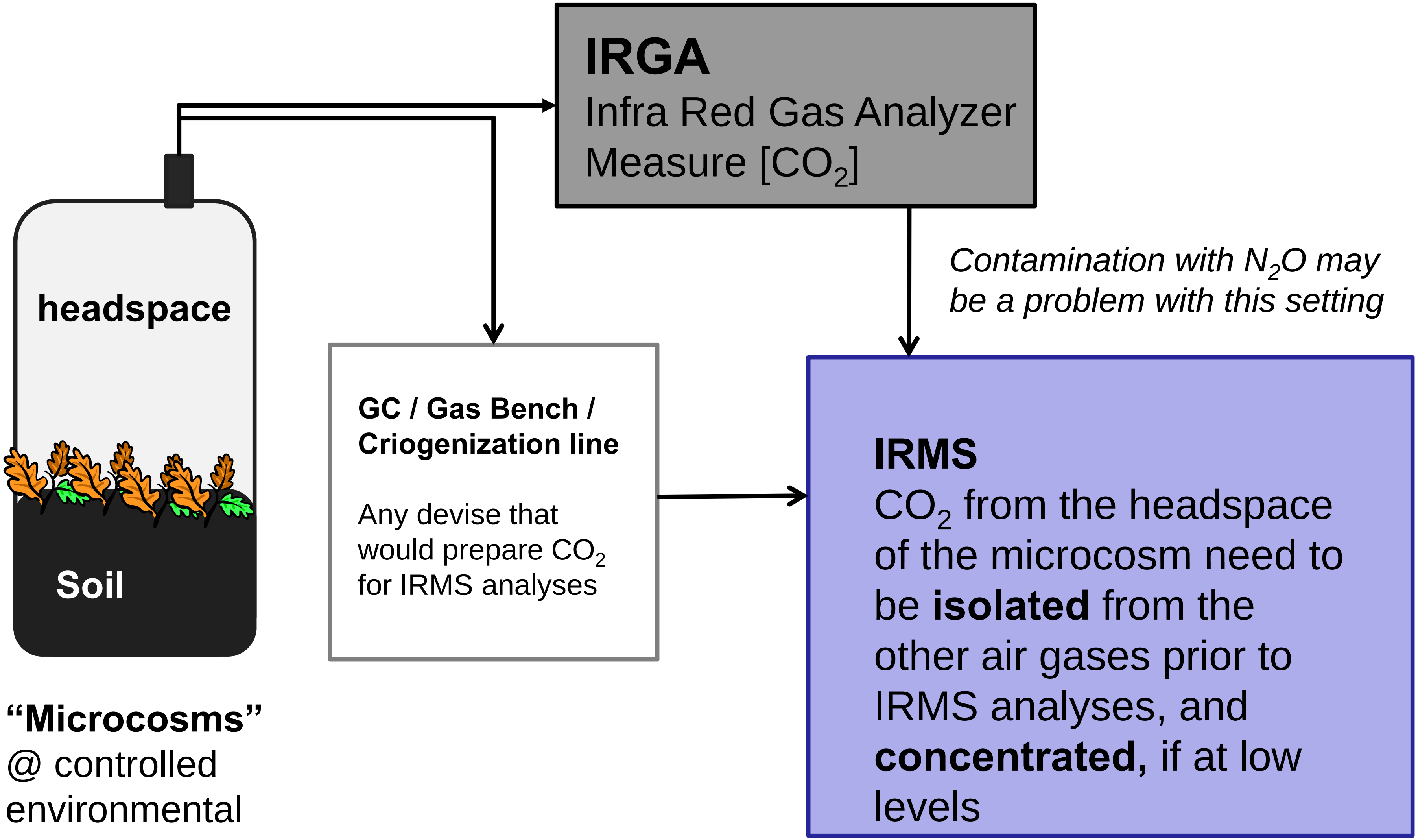
$$c_{litter} = \delta_{t} - \delta_{soil} / \delta_{litter} - \delta_{soil}$$

$$C_{litter} = c_{litter} * C_{t}$$

$$C_{loss} = C_{litter} + F_{litter}$$

CO_2 efflux and ^{13}C of CO_2 need to be measured … HOW??

Experimental setting



IRGA
Infra Red Gas Analyzer
Measure [CO_2]

Contamination with N_2O may be a problem with this setting

GC / Gas Bench / Criogenization line

Any devise that would prepare CO_2 for IRMS analyses

IRMS
CO_2 from the headspace of the microcosm need to be **isolated** from the other air gases prior to IRMS analyses, and **concentrated,** if at low levels

"Microcosms"
@ controlled environmental conditions

Which δ^{13}C is best to use as end-members?

Some fractionation may occur during heterotrophic respiration, for this reason <u>it is advisable to use the δ^{13}C respired by the two sources</u>, generally measured in *ad hoc* incubations, rather than the δ^{13}C of the source it self (i.e. bulk litter or SOM).

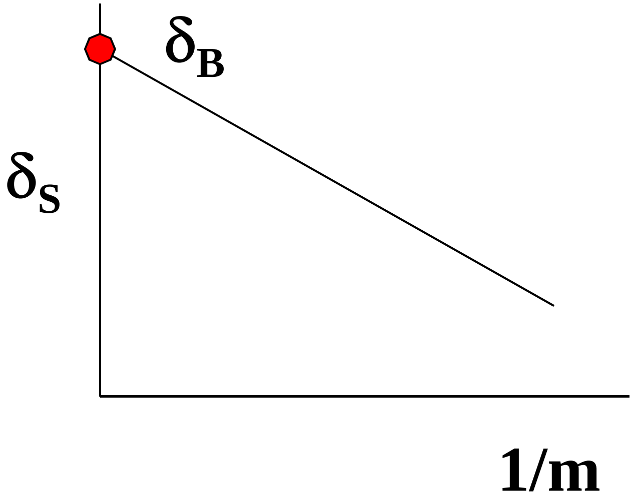
However, the use of the δ^{13}C of the source, is generally accepted in the literature (provided that the assumption of no fractionation is stated in the method)

Can we use the linear mixing model to quantify the isotopic signal of a CO_2 source?

If we know:

- ✓ the δ of the mixture,
- ✓ and its mass,

applying the mass balance equation and rewriting:



$$m_S = m_A + m_B$$

$$\delta_S m_S = \delta_A m_A + \delta_B m_B$$

$$\boldsymbol{\delta_S = \delta_B + (1/m_S * m_A * (\delta_A - \delta_B)}$$

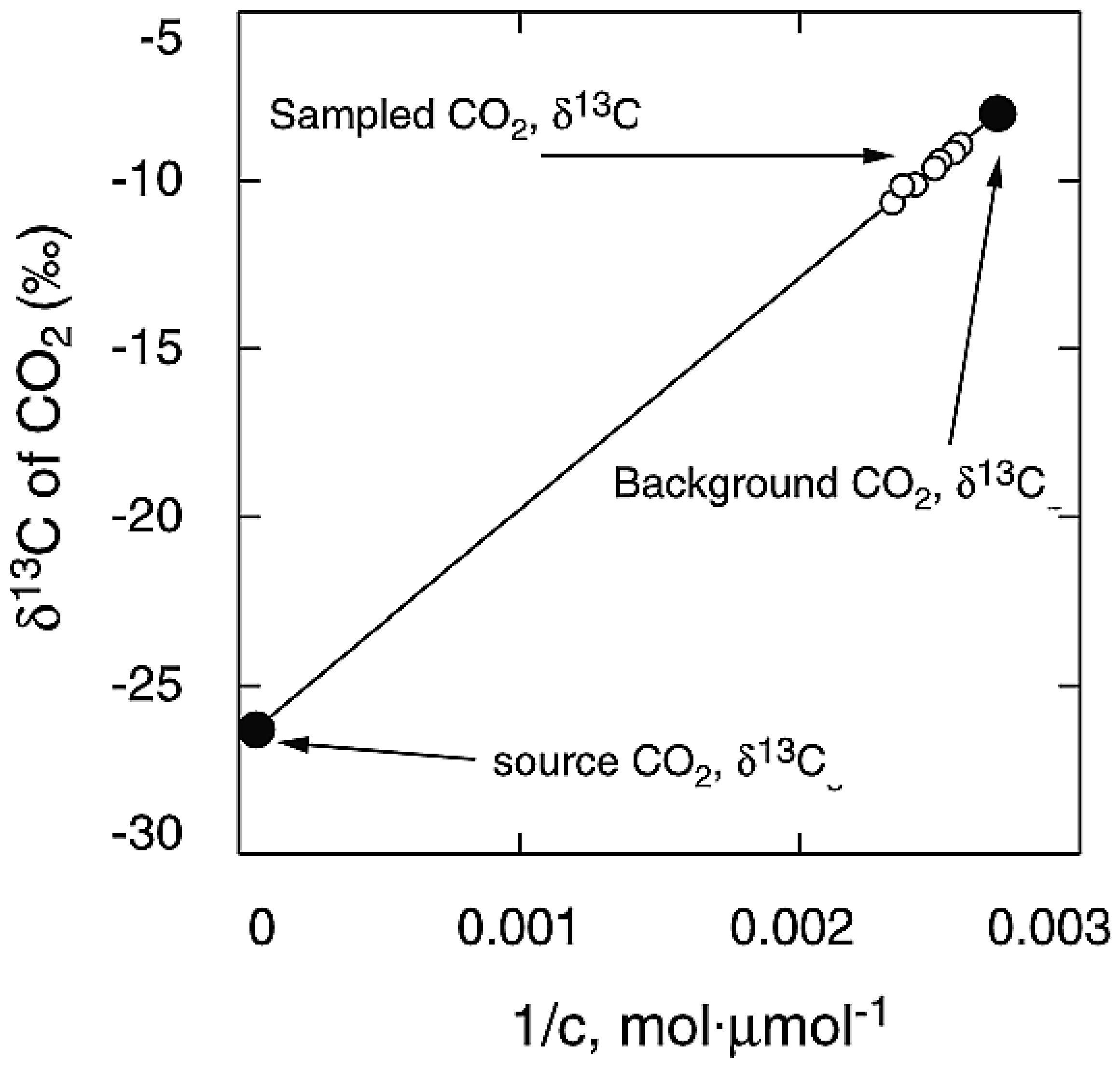
$$\boldsymbol{Y = a + 1/x * b}$$

For example ….

- We can quantify the $\delta^{13}C$ of the ecosystem respired CO_2 by measuring the $\delta^{13}C$-CO_2 and the CO_2 concentration at night along a large enough range (i.e. about 100 ppm) of $[CO_2]$.

- This is called the **Keeling Plot**, after Keeling (1953) who first proposed it.

Keeling plot



Pataki et al. 2003

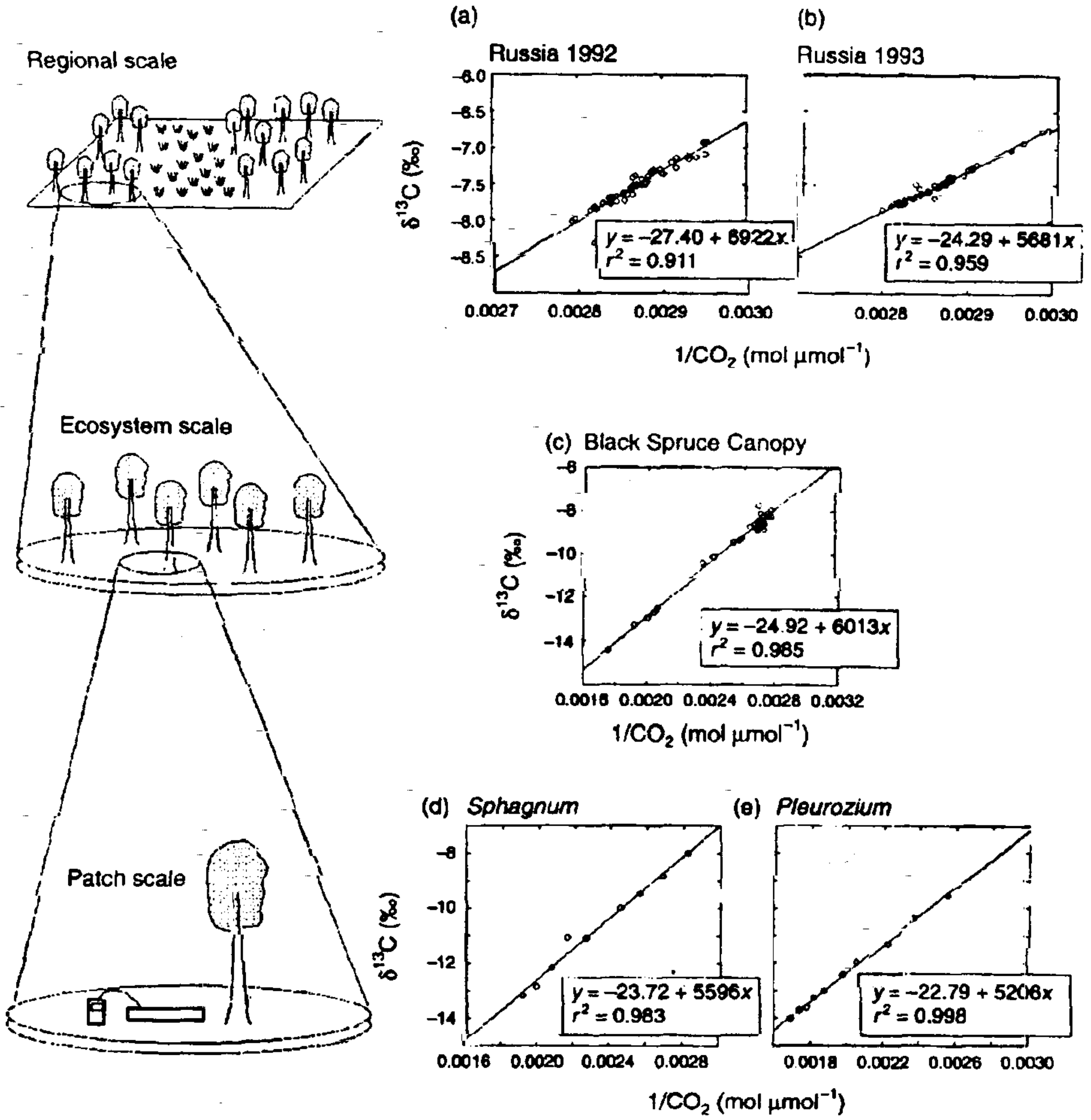
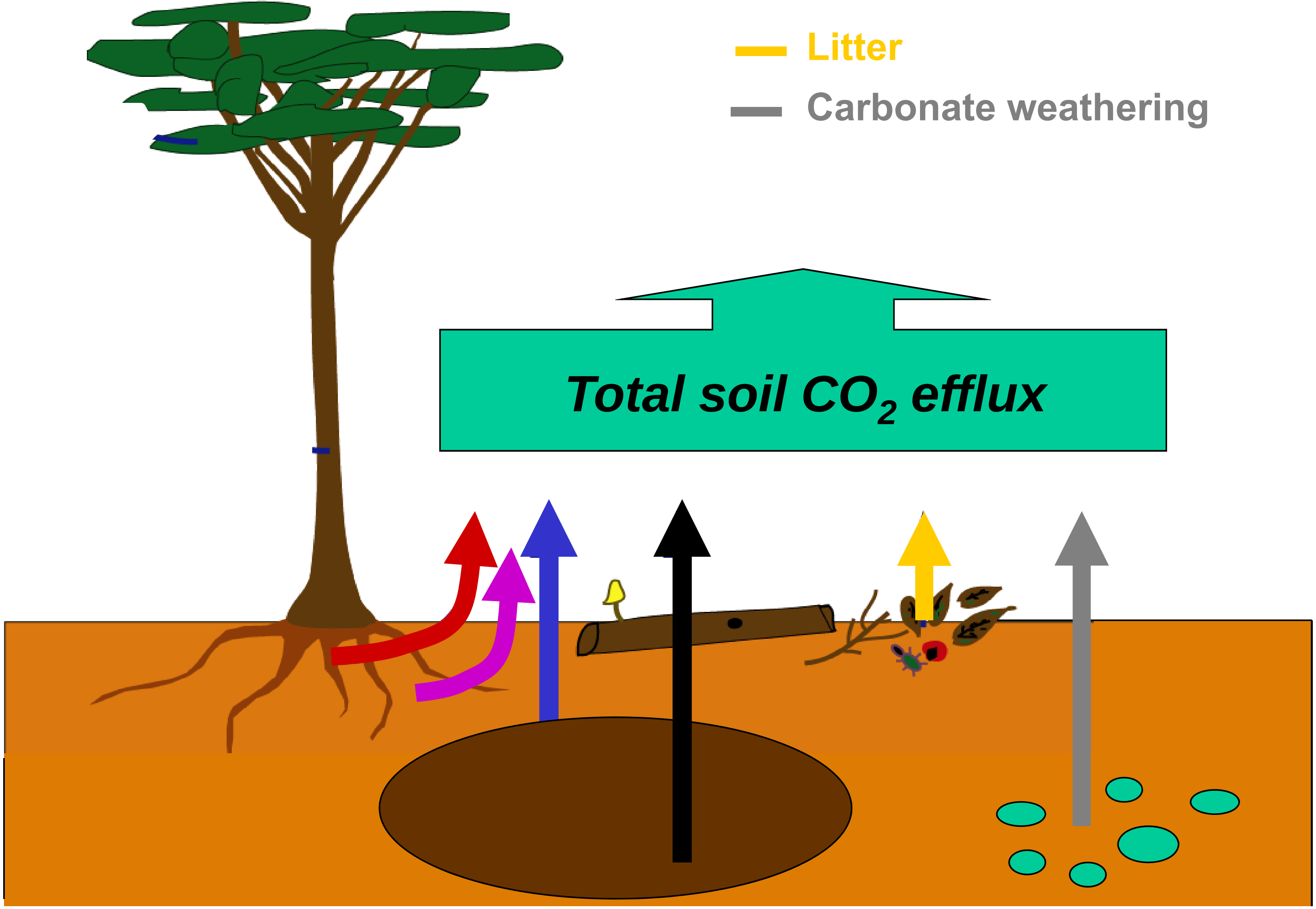


Fig. 3. Comparison of the carbon isotope ratio of respired CO_2 at different spatial scales, from analysis of the relationship between 1/CO_2 concentration and the carbon isotope ratio of atmospheric CO_2 (see Box 1 for the theory behind the technique). Aircraft air sample data (a,b) are from Ref. 29. The forest floor (d,e) and canopy (c) data come from samples collected in a black spruce forest in northern Canada (see Ref. 20).

Partitioning soil respiration in the field

Soil CO$_2$ efflux components

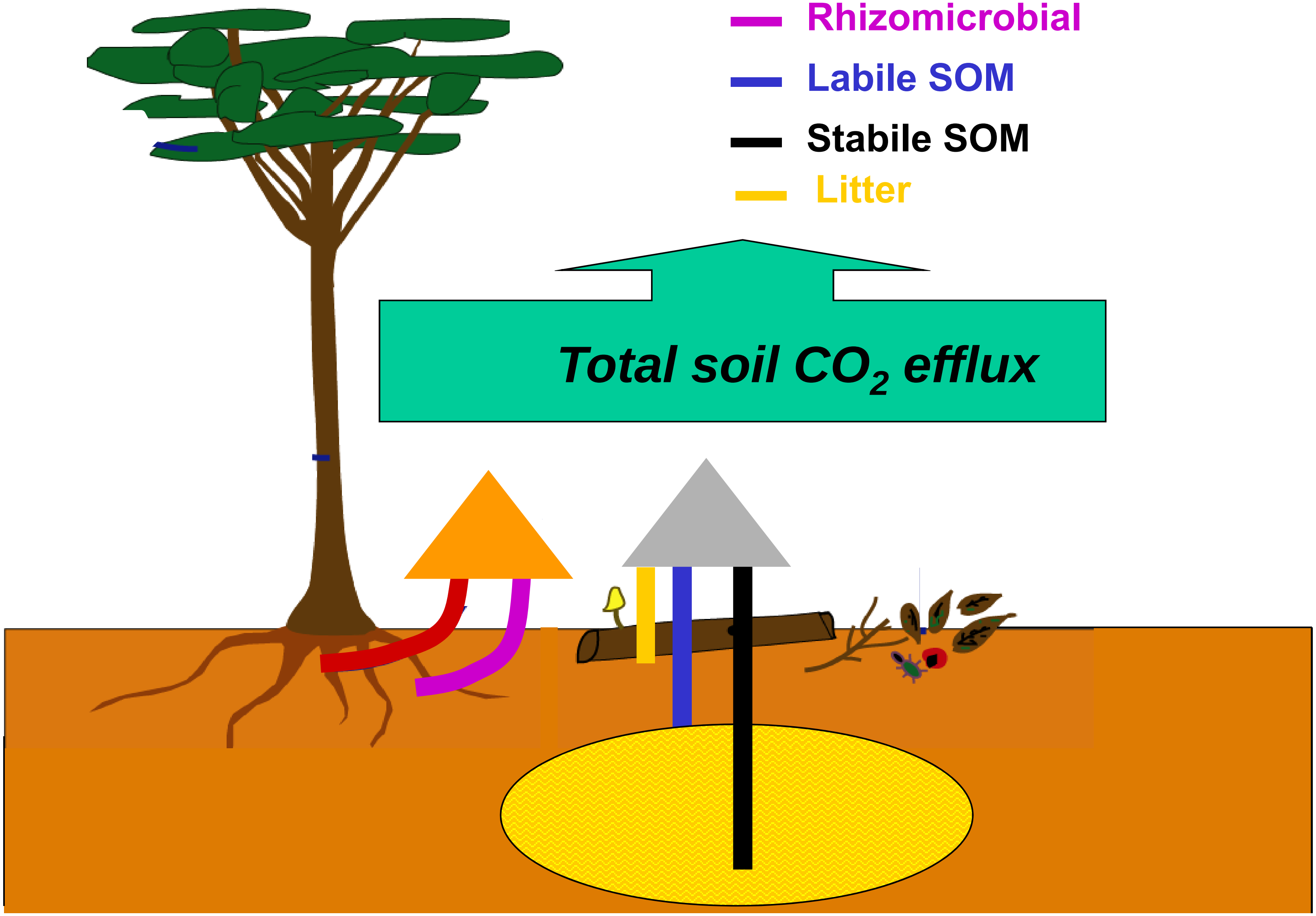
- ▬ **Root growth and maintenance**
- ▬ **Rhizomicrobial**
- ▬ **Labile SOM**
- ▬ **Stabile SOM**
- ▬ **Litter**
- ▬ **Carbonate weathering**



Total soil CO$_2$ efflux

Soil CO_2 efflux components: identification and meaning

- ▬ Autotrophic
- ▬ Heterotrophic
- ▬ Root growth and maintenance
- ▬ Rhizomicrobial
- ▬ Labile SOM
- ▬ Stabile SOM
- ▬ Litter



Measuring soil CO_2 and $d^{13}CO_2$ in the field

- The most common way to measure CO_2 efflux is by dynamic closed chambers connected to a IRGA. The system can be automated for continuous measurement in the field.
- Measurements of pCO_2 in the soil air along the soil profile and modeling of efflux are also used.
- Soil temperature and humidity should be recorded at time of measurement

- For the measurement of $^{13}CO_2$, most commonly CO_2 is let to accumulate overtime in the headspace of a closed static chamber and sub samples of air withdrawn periodically and analyzed for $[CO_2]$ and $^{13}CO_2$. The $\delta^{13}C$ of soil respiration is obtained by a Keeling plot
- Alternatively, only one sample is collected after sufficient accumulation of CO_2, and analyzed for $[CO_2]$ and $^{13}CO_2$. The $\delta^{13}C$ of soil respiration is obtained by a mass balance approach, provided that the $\delta^{13}C$ of atmospheric CO_2 is known.

$$\delta^{13}C_{resp} = [(\delta^{13}C_{ch} * C_{ch}) - (\delta^{13}C_{atm} * C_{atm})] / C_{resp}$$

- Measurements of $^{13}CO_2$ in the soil air along the soil profile and modeling of efflux are also used.

Partitioning autotrophic *vs* heterotrophic respiration:

planting C4 plants in C3 soil or viceversa

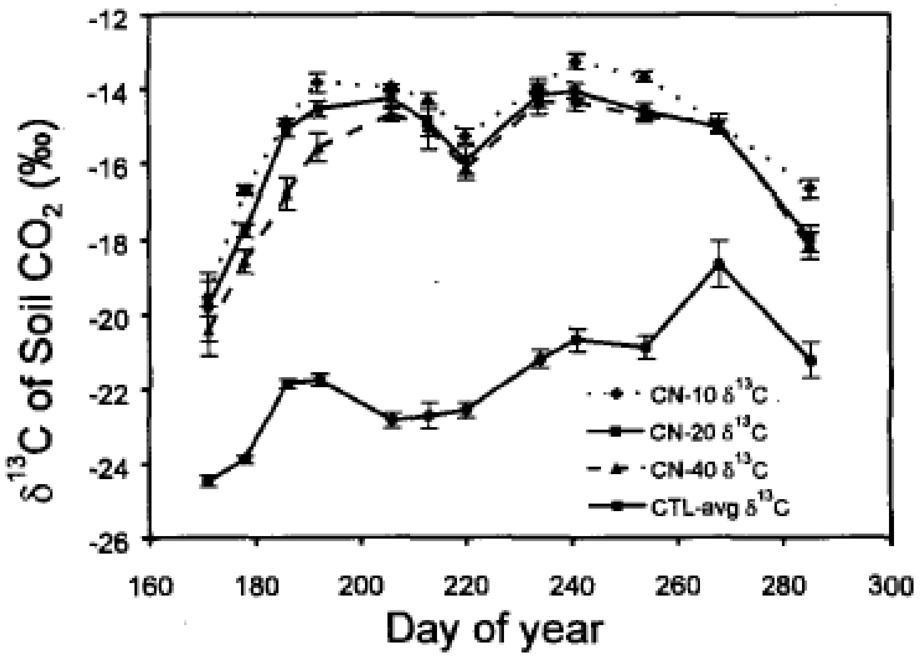


Fig. 2. Seasonal variation in the C isotope ratio of soil CO_2 in corn and control plots. Values for the control plot represent averages for samples taken at the three different soil depths. Error bars = ±SE.

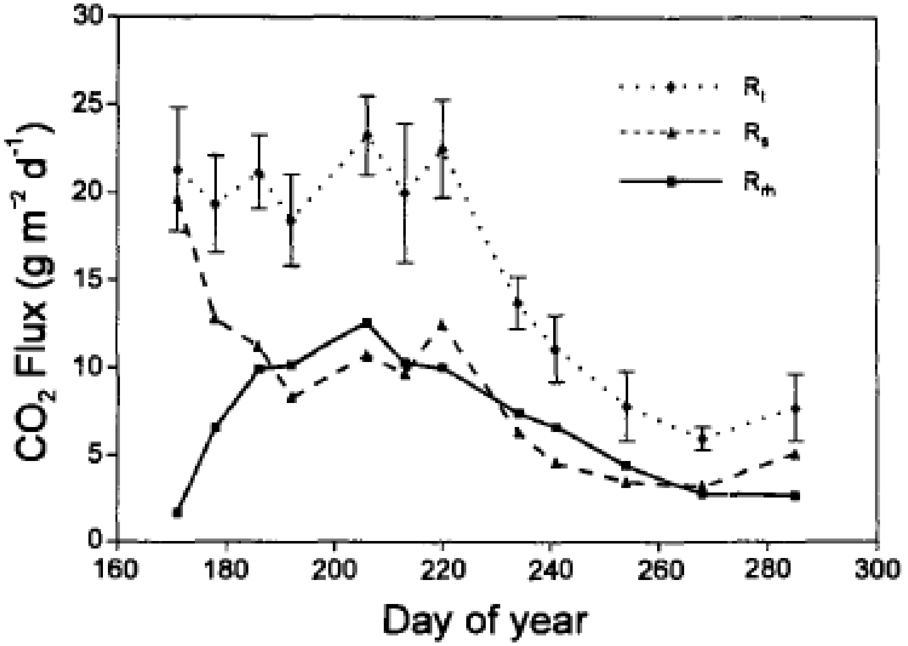


Fig. 5. Total soil (R_t), rhizosphere (R_{rh}), and soil (R_s) respiration in a corn crop during the 1995 growing season in Ottawa, Canada. Vertical bars indicate ±SD.

Rochette & Flanagan, 1997

Partitioning autotrophic *vs* heterotrophic respiration: using the ^{13}C depleted signal in FACE studies

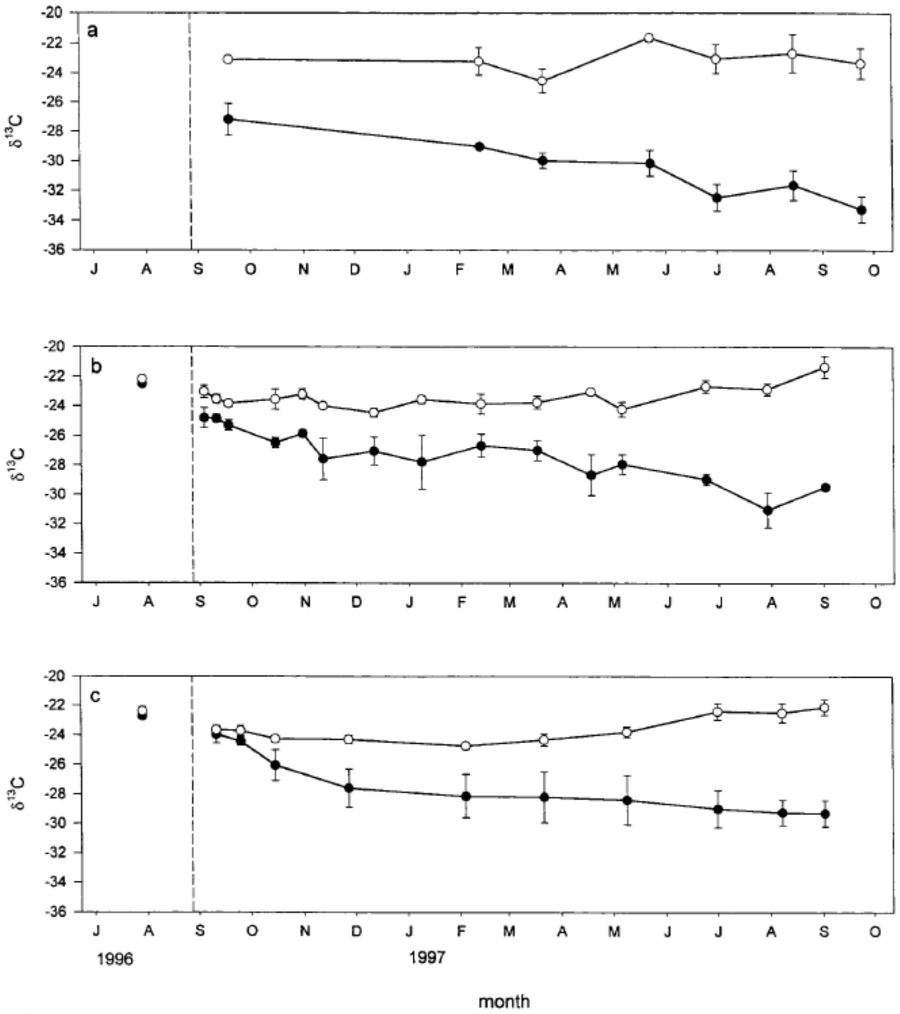


Fig. 1. The δ^{13}C of CO_2 from (a) surface flux, (b) 15-cm depth, and (c) 30-cm depth collected from the replicated FACE experiment in treatment (closed circles, $n = 3$) and control (open circles, $n = 3$) plots. Error bars show 1 standard error from the mean. Vertical broken lines show the start of CO_2 fumigation.

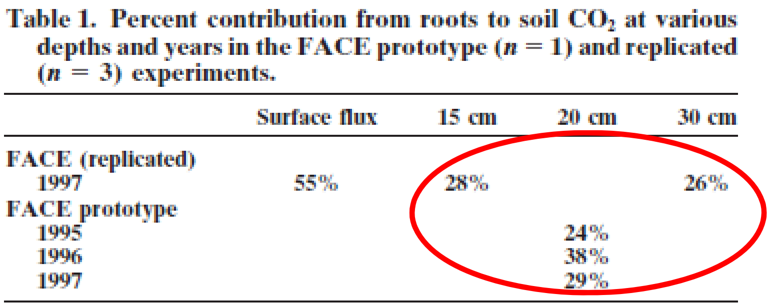


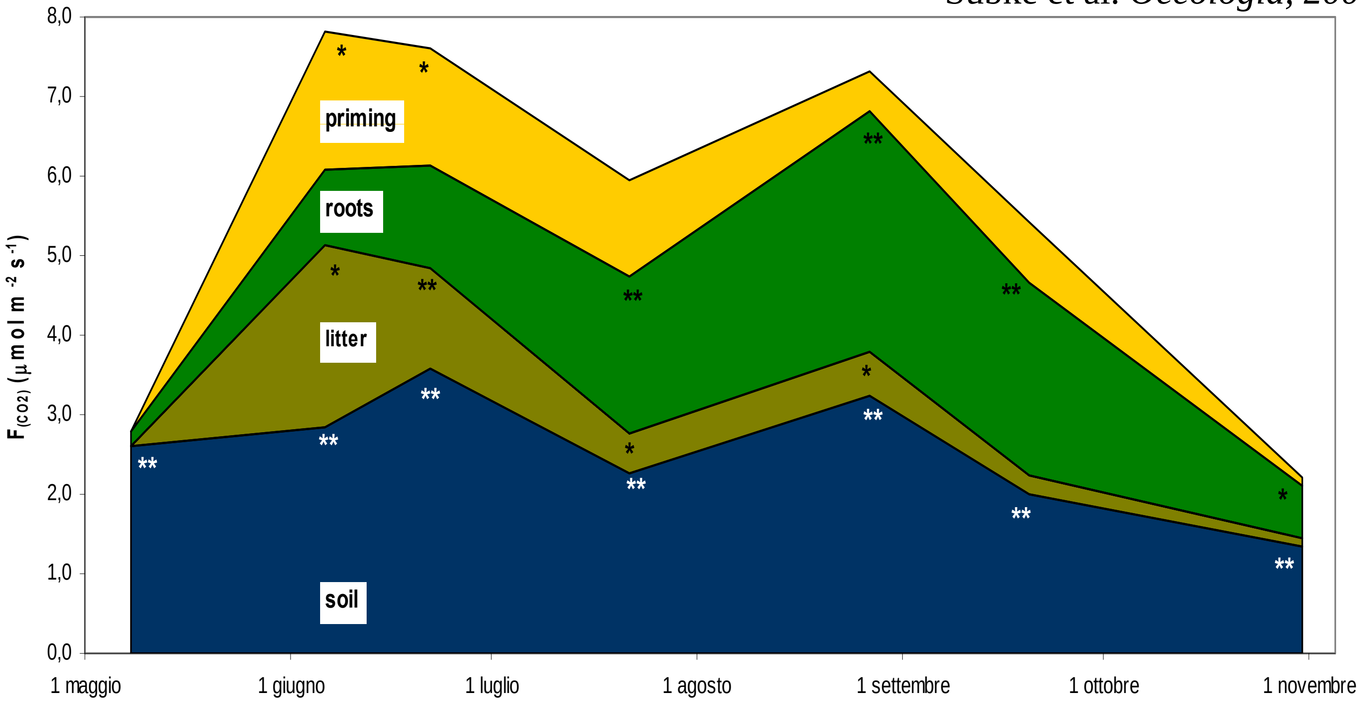
Table 1. Percent contribution from roots to soil CO_2 at various depths and years in the FACE prototype ($n = 1$) and replicated ($n = 3$) experiments.

	Surface flux	15 cm	20 cm	30 cm
FACE (replicated)				
1997	55%	28%		26%
FACE prototype				
1995			24%	
1996			38%	
1997			29%	

Andrews et al., 1999

Partitioning multiple sources: a combination of isotopic and other approaches

Subke et al. *Oecologia, 2004*



Girdling experiments

Girdling = cut bark, phloem until cambium

↓

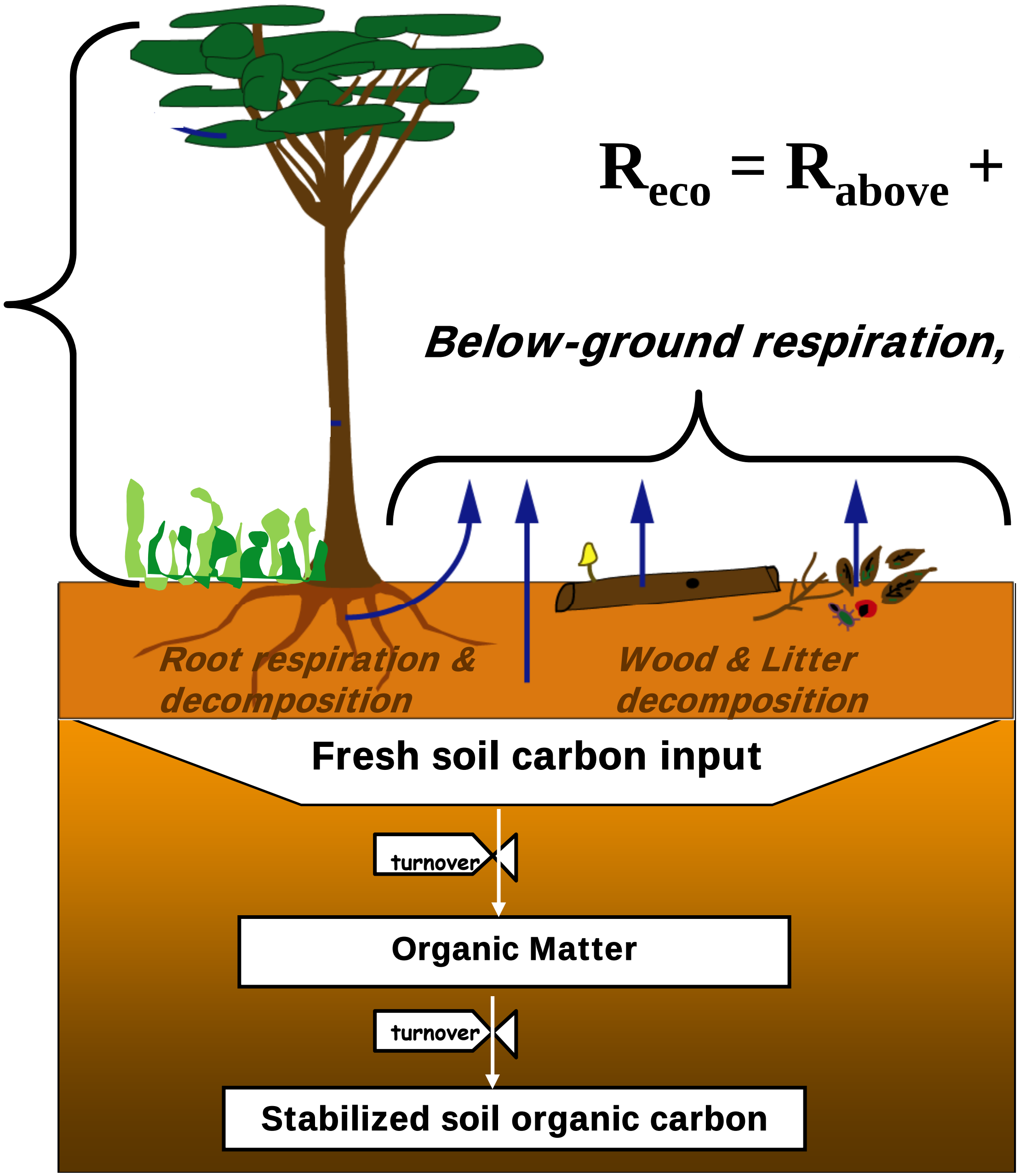
Roots are not supplied with photosynthetates

↓

Root respiration is inhibited

ECOSYSTEM RESPIRATION (R_{eco})

$$R_{eco} = R_{above} + R_{below}$$



Above ground respiration

R_{above}

Below-ground respiration, R_{below}

Root respiration & decomposition

Wood & Litter decomposition

Fresh soil carbon input

turnover

Organic Matter

turnover

Stabilized soil organic carbon

Tu & Dawson,2005

Partitioning ecosystem respiration

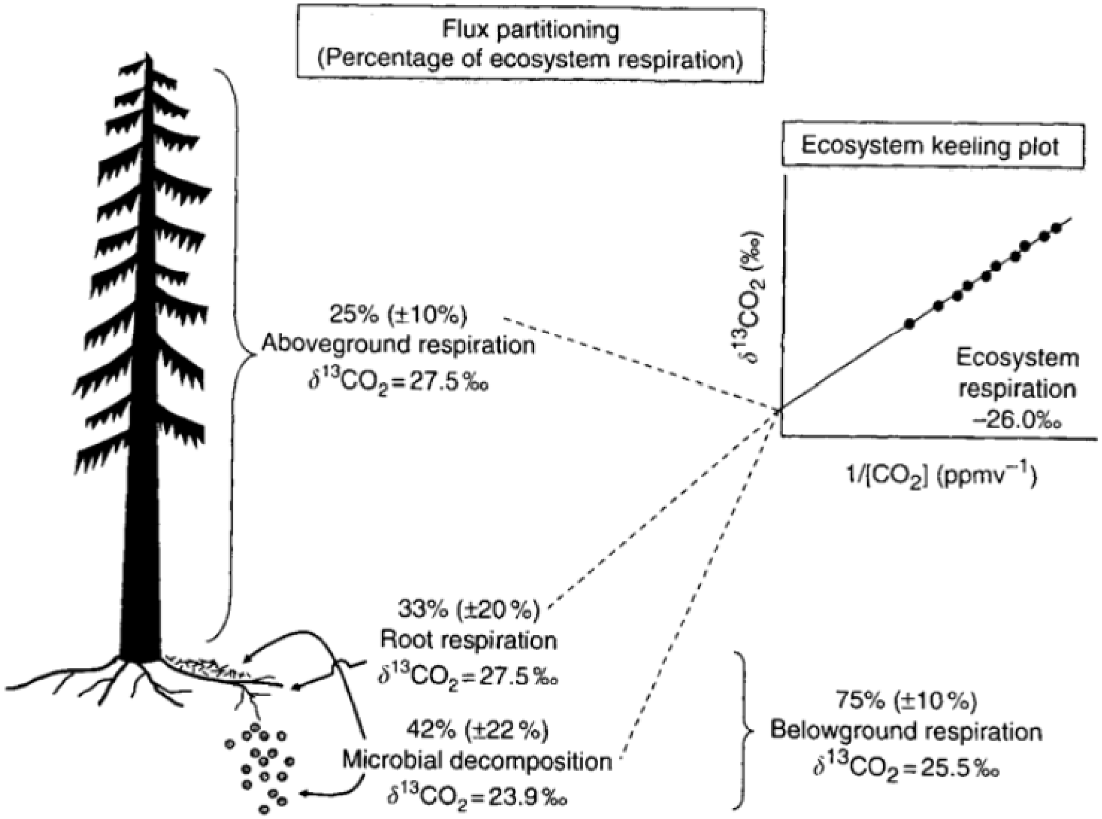


Figure 8.13 Partitioning ecosystem respiration using stable carbon isotope signatures. Samples were collected in a redwood forest (*Sequoia sempirvirens*) near Occidental, California (unpublished results) using the syringe method described in the section Determining Source Signatures for determining the isotopic signature of aboveground (leaf), root, and microbial respiration. The Keeling plot method was used for belowground and ecosystem respiration by sampling air within a chamber on the soil surface and from within the canopy airspace at night, respectively. Note that roots and microbes contribute ~44% and ~56% to belowground respiration respectively (root = 33/75, microbial = 42/75). The values in parentheses are the standard errors of the partitioning estimates as determined following Phillips and Gregg (2001).

Tu&Dawson, 2005

Variation in the stable C isotope natural abundance is often too little to use in studies of soil C dynamics.

What can we do then?

Rare Isotope Enrichment

Isotope labeling

Consists in the addition of large amounts of the rare isotope to a pool of interest (i.e. CO_2, NH_4^+ , etc.)

- **to trace** plant-soil C&N flows, nutrient allocation, foodweb studies, biomarkers, etc.

Requires a good knowledge of the system under study and of a good model to interpret the data!

<u>Fractionation can be neglected !</u>

Isotope labeling

1. Labeling plants with $^{13}CO_2$ or $^{14}CO_2$:
   Continuous *vs* Pulse labeling

2. Amendments of ^{13}C or ^{14}C labeled substrates

3. Addition of labeled (i.e. ^{15}N, ^{32}P, ^{18}O or ^{2}H enriched) mineral nutrients (i.e. NH_4^+, NO_3^-, PO_4^{2-}, etc.)

4. Addition of labeled (i.e. ^{18}O or ^{2}H enriched) water

^{13}C PULSE LABELING:

Closed labeling chamber

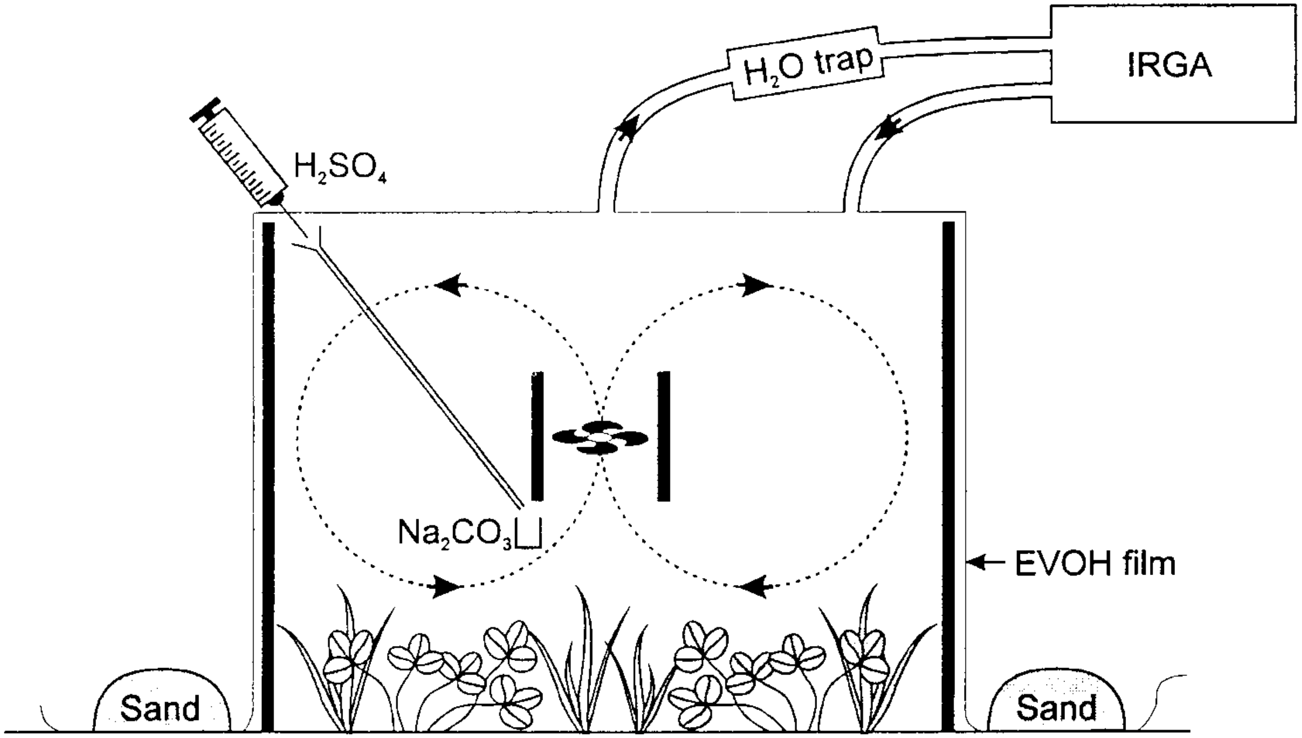
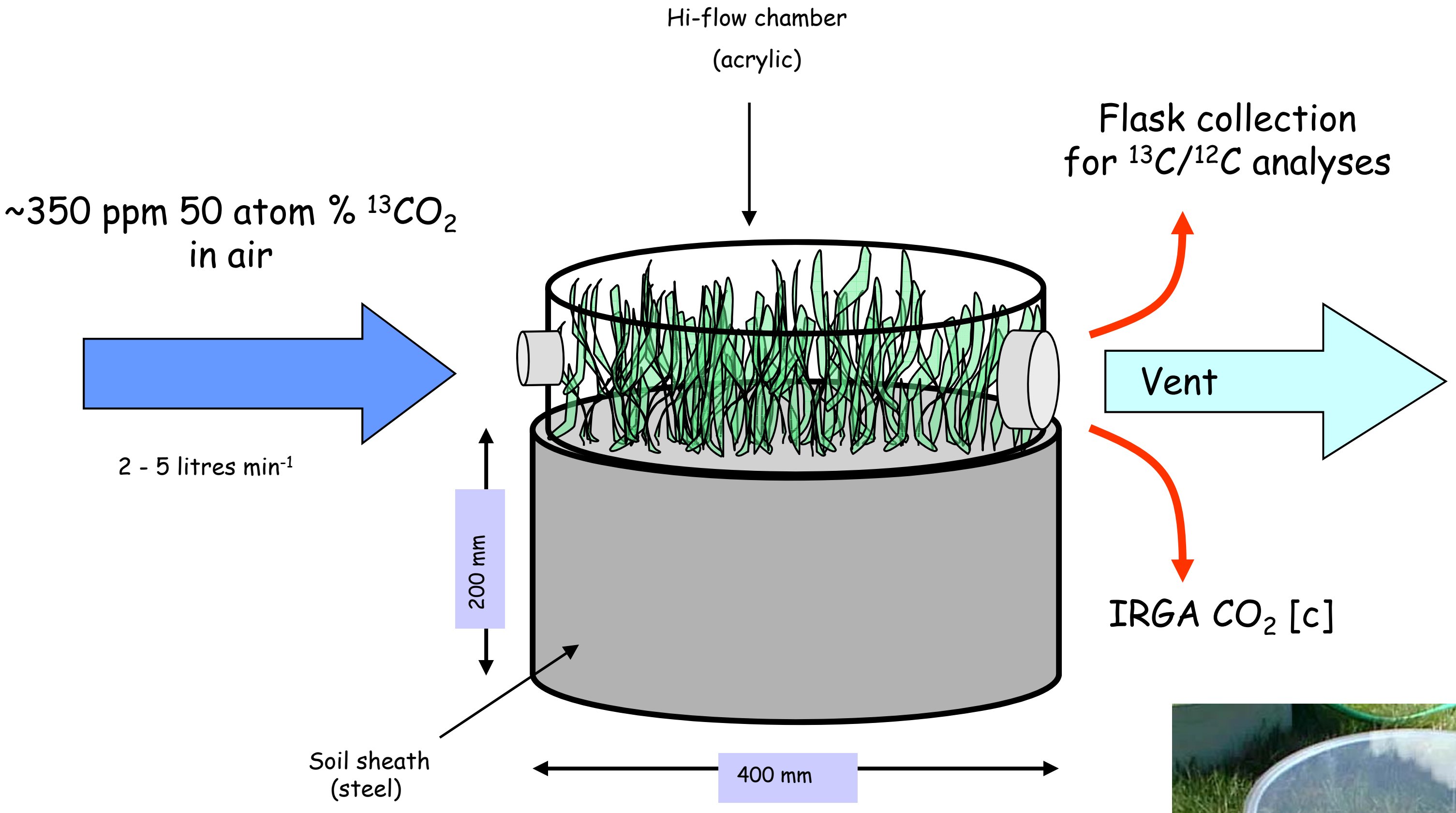


Figure 1. Layout of the canopy, infra-red gas analyser (IRGA) and other equipment during labelling (see text for description).

Stewart & Metherell (1999), *Plant & Soil* 210, 61-73

^{13}C PULSE LABELING:

Isotope labeling flow-through chamber



Hi-flow chamber
(acrylic)

Flask collection
for $^{13}C/^{12}C$ analyses

~350 ppm 50 atom % $^{13}CO_2$
in air

Vent

2 - 5 litres min^{-1}

200 mm

IRGA CO_2 [c]

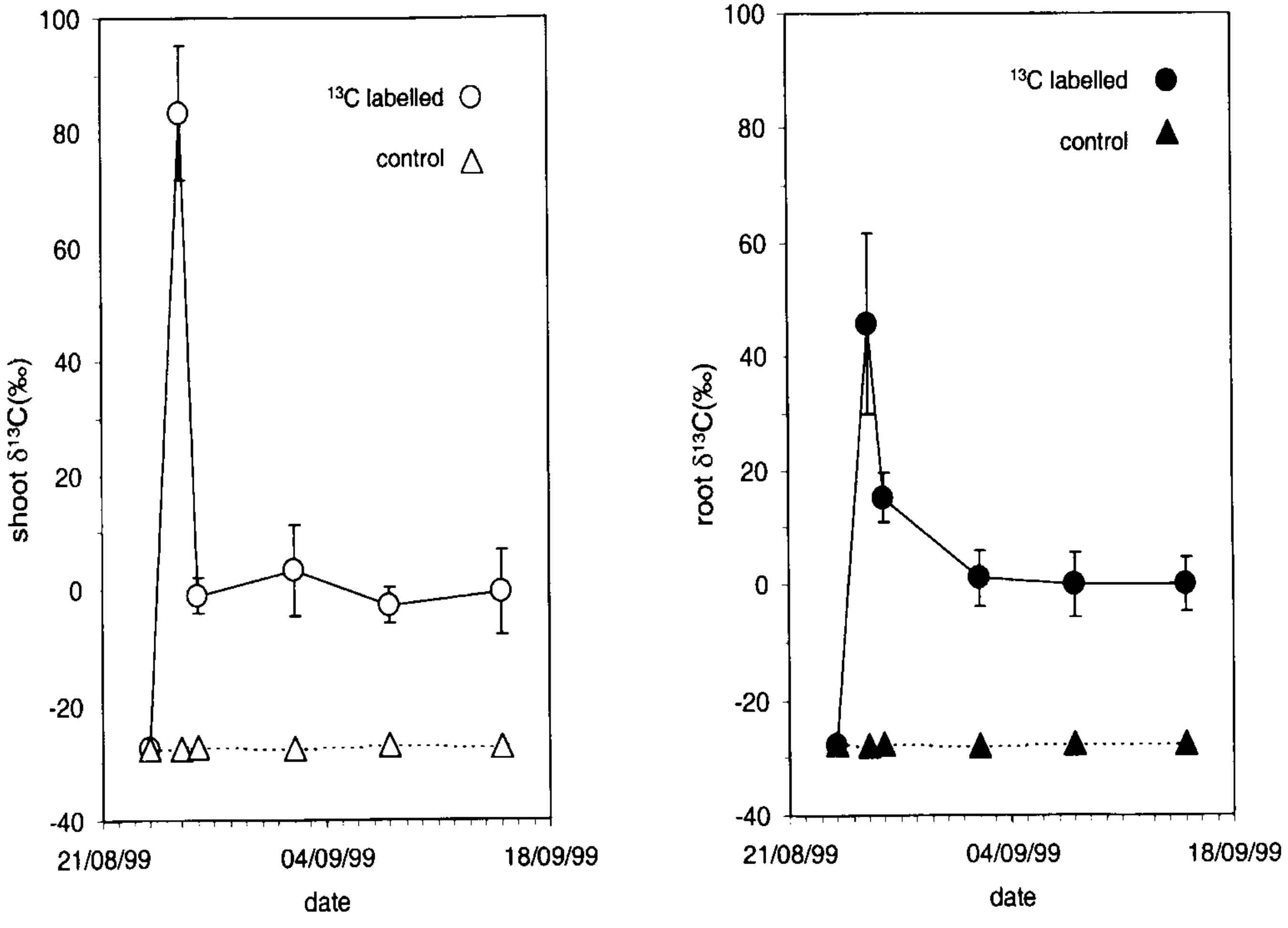
Soil sheath
(steel)

400 mm

Soil
Biodiversity
NERC Thematic Programme

^{13}C PULSE LABELING:

Time curse of the pulse ^{13}C label in shoot and root



Ostle et al., 2000

^{13}C CONTINUOUS LABELING:

Closed labeling chamber

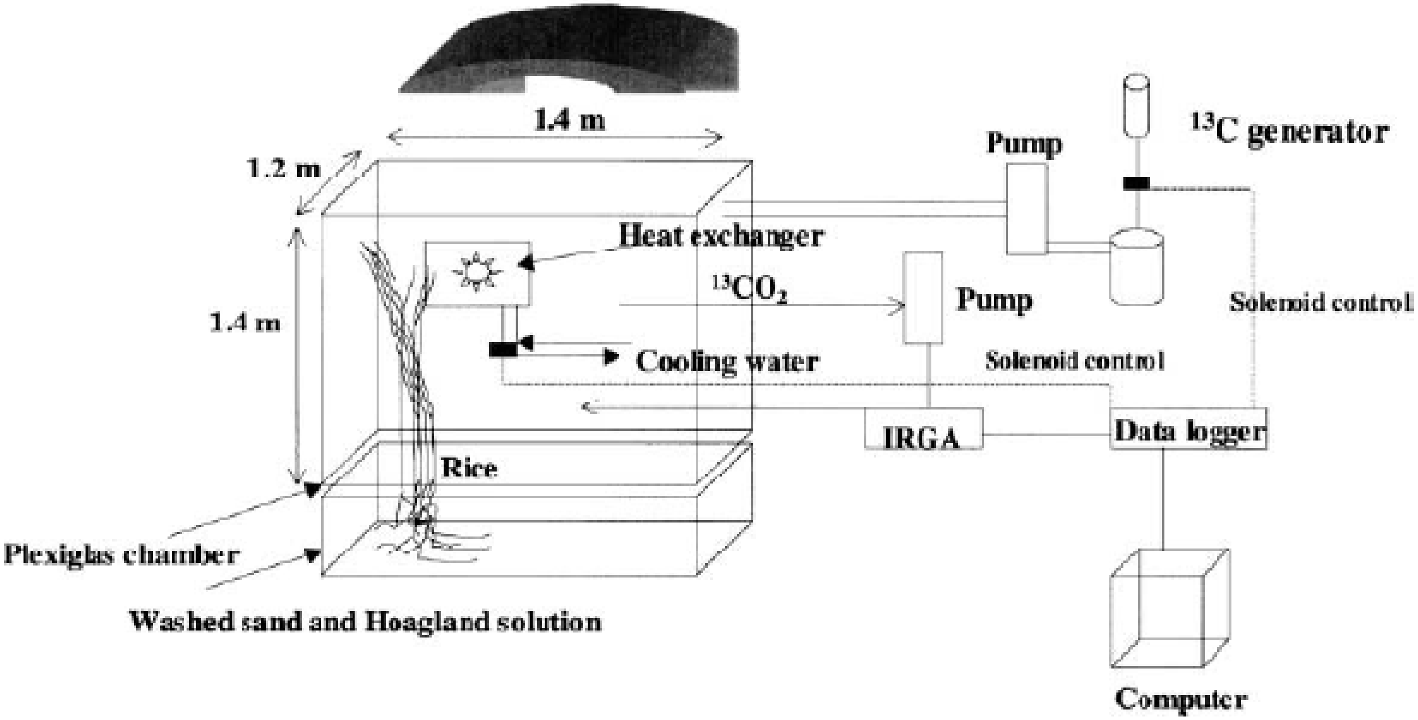


Fig. 2. Design of stable isotope ^{13}C- and ^{15}N-labeling growth chamber. Two square meters of rice (cultivar M-103) was produced in greenhouse facilities at the University of California, Davis.

Bird et al., 2003

^{13}C CONTINUOUS LABELING:

Our Closed labeling chamber

An example of amendments with a ^{13}C labeled plant material produced by continuous isotope labeling of poplar seedlings in a growth chamber

Incubation of ^{13}C enriched leaf litter at the POP_EUROFACE experimental site

Quantifying the relative contribution of above ground leaf litter to soil respiration

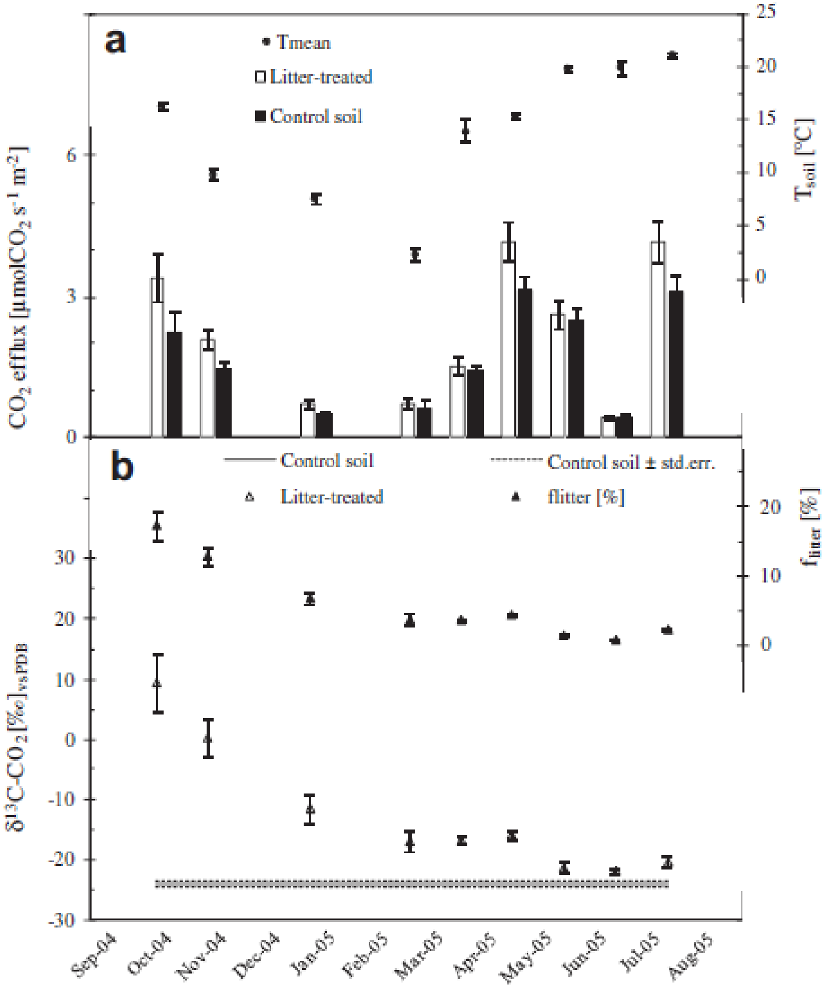


Fig. 1. a) monthly respiration rate [µmol CO$_2$ s^{-1} m^{-2}] for litter-treated (white bars) and control soils (black bars). Black circles represent mean soil temperature on each sampling day; b) δ^{13}C–CO$_2$ [‰]$_{vsPDB}$ of litter-treated (white triangles) and control soil (black line) and fraction (black triangles) of litter-derived C in the CO$_2$ respired by litter-treated soil. Error bars are standard errors over $n = 12$ replicates.

By applying the mixing model the relative contribution (f) of above ground litter to total soil respiration can be calculated

$$\mathbf{f_{litter} = \delta_{total} - \delta_{soil} / \delta_{litter} - \delta_{soil}}$$

When planning this kind of experiment it is important to remember to be able to measure all the δ values of the ***end members*** *to solve the mixing model!*

Rubino et al., 2010

Tracing litter-derived C along the soil profile

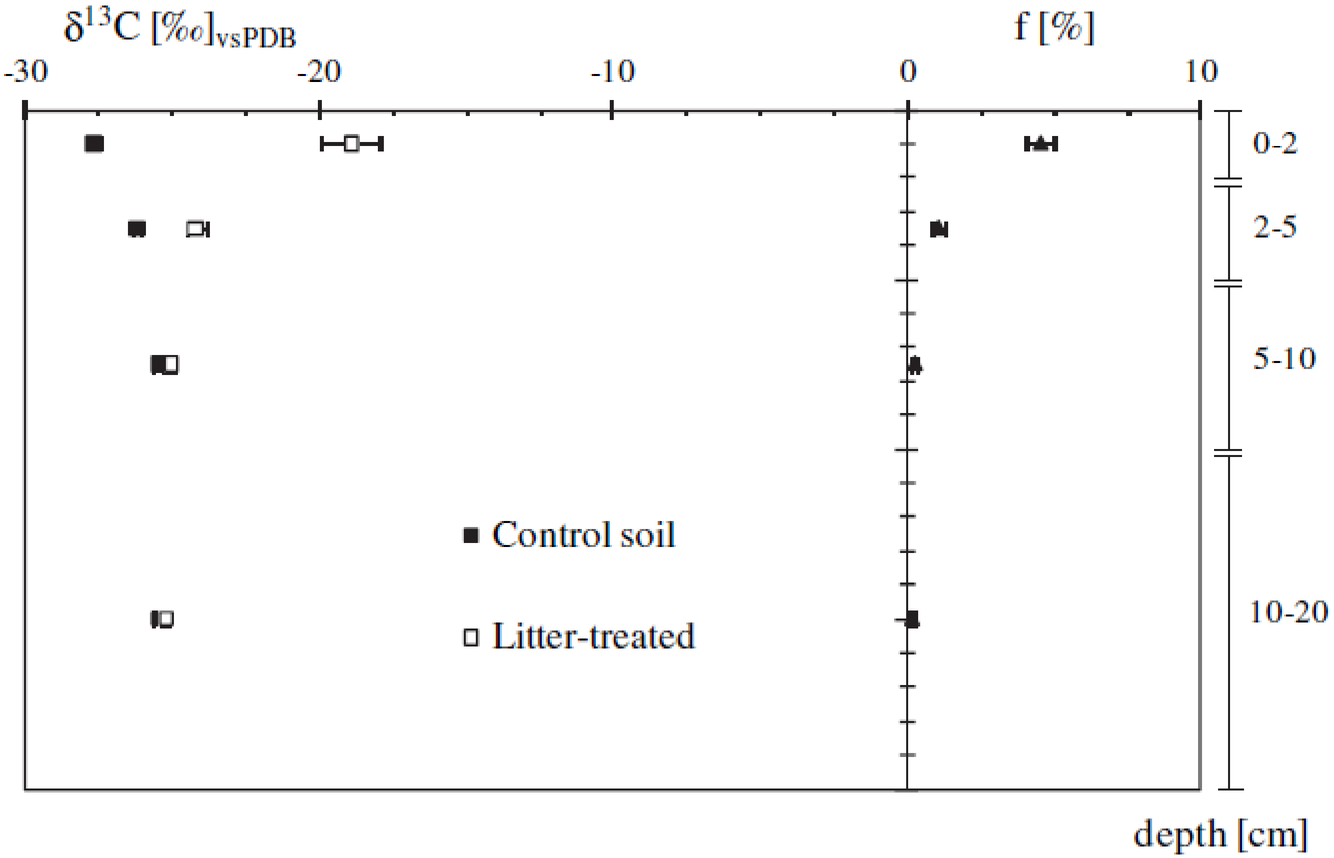
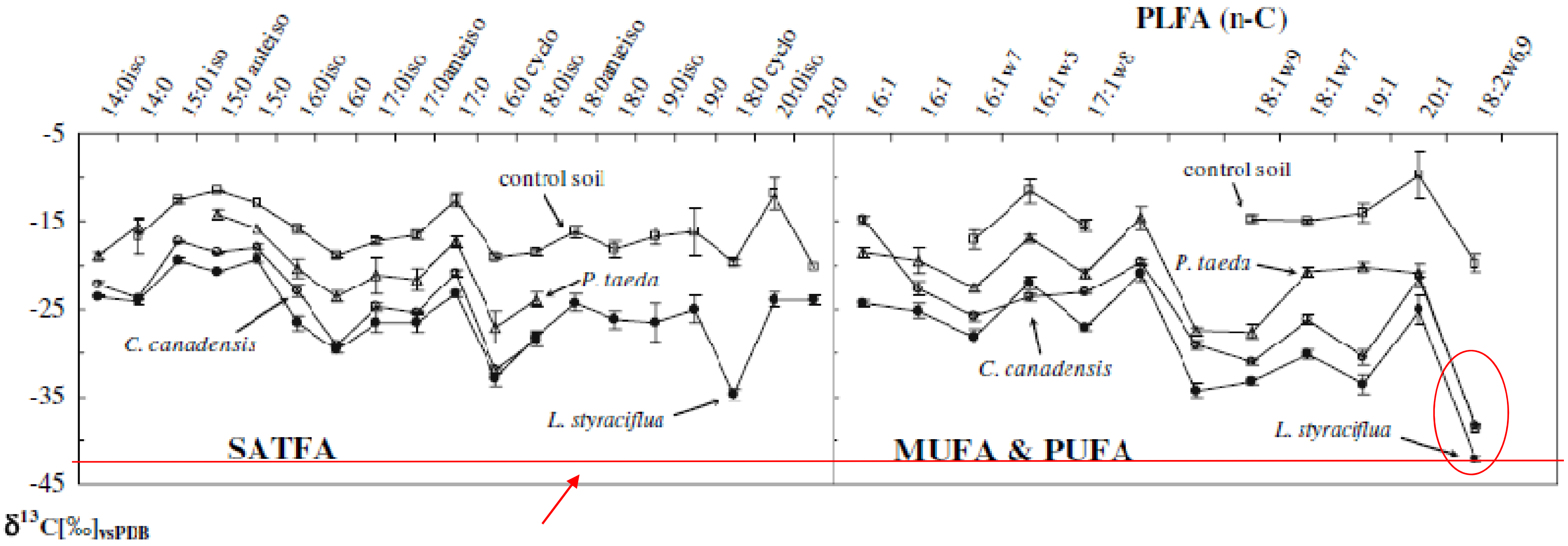


Fig. 2. $\delta^{13}C{-}CO_2$ [‰]$_{vsPDB}$ (left side) of litter-treated soil (white squares) and control soils (black squares) and fraction (%, right side) of litter-derived C in soil for each sampled soil layer (0–2, 2–5, 5–10, 10–20 cm). Error bars are standard errors over 12 replicates.

Rubino et al., 2010

Identifying organisms specifically linked to functions



Plant litter
$\delta^{13}C$

Rubino et al., 2009

Isotope labeling of biomarkers,

in microbial ecology (Boschker & Middelburg, 2002; Leakes et al., 2006)

Biomarkers are compounds that provide information on microbial identity and possibly on biomass (i.e. PLFAs, rRNA, etc.)

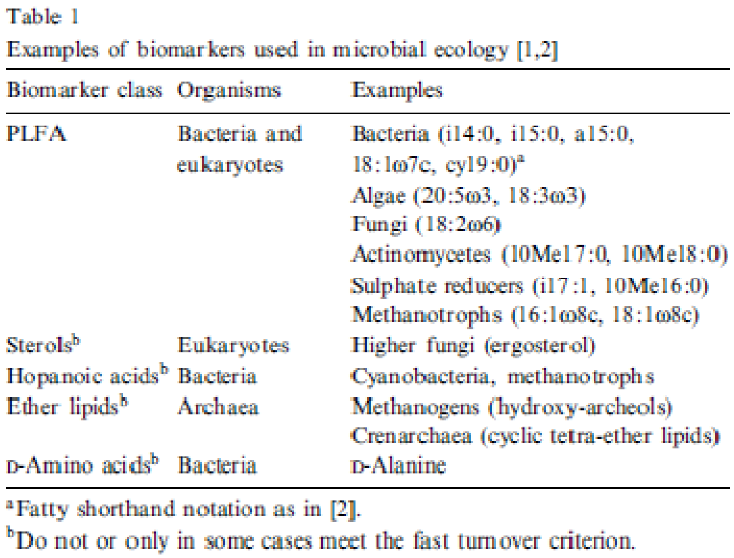


Table 1

Examples of biomarkers used in microbial ecology [1,2]

Biomarker class	Organisms	Examples
PLFA	Bacteria and eukaryotes	Bacteria (i14:0, i15:0, a15:0, 18:1ω7c, cy19:0)[a] Algae (20:5ω3, 18:3ω3) Fungi (18:2ω6) Actinomycetes (10Me17:0, 10Me18:0) Sulphate reducers (i17:1, 10Me16:0) Methanotrophs (16:1ω8c, 18:1ω8c)
Sterols[b]	Eukaryotes	Higher fungi (ergosterol)
Hopanoic acids[b]	Bacteria	Cyanobacteria, methanotrophs
Ether lipids[b]	Archaea	Methanogens (hydroxy-archeols) Crenarchaea (cyclic tetra-ether lipids)
D-Amino acids[b]	Bacteria	D-Alanine

[a] Fatty shorthand notation as in [2].

[b] Do not or only in some cases meet the fast turnover criterion.

Labeling of biomarkers allow for direct links of specific microbial groups to functions

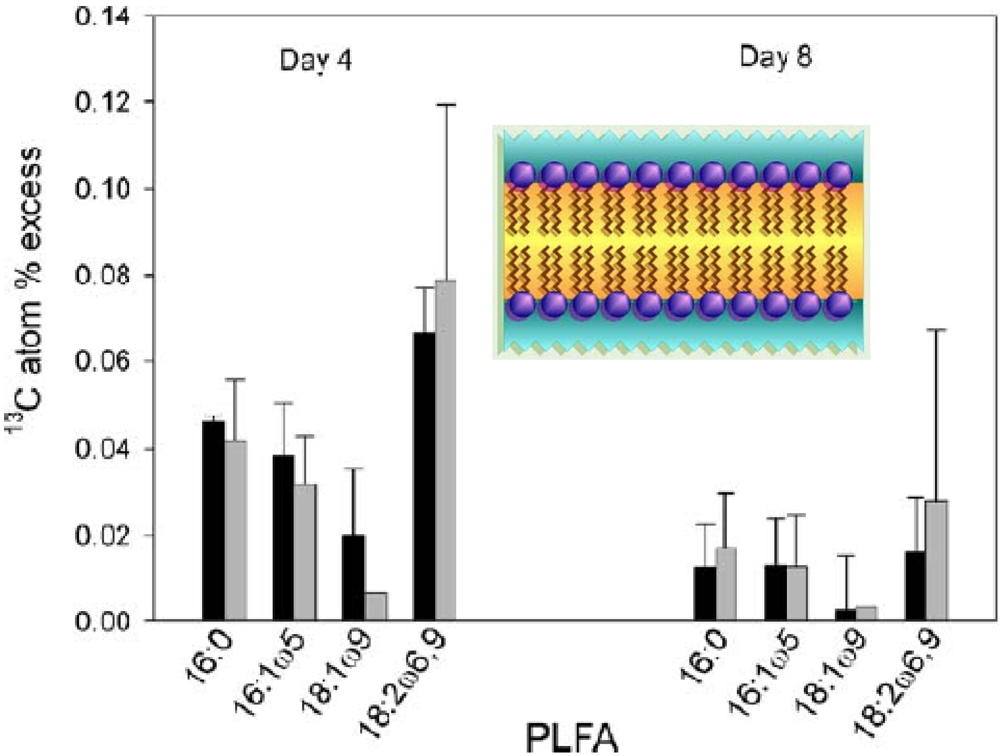
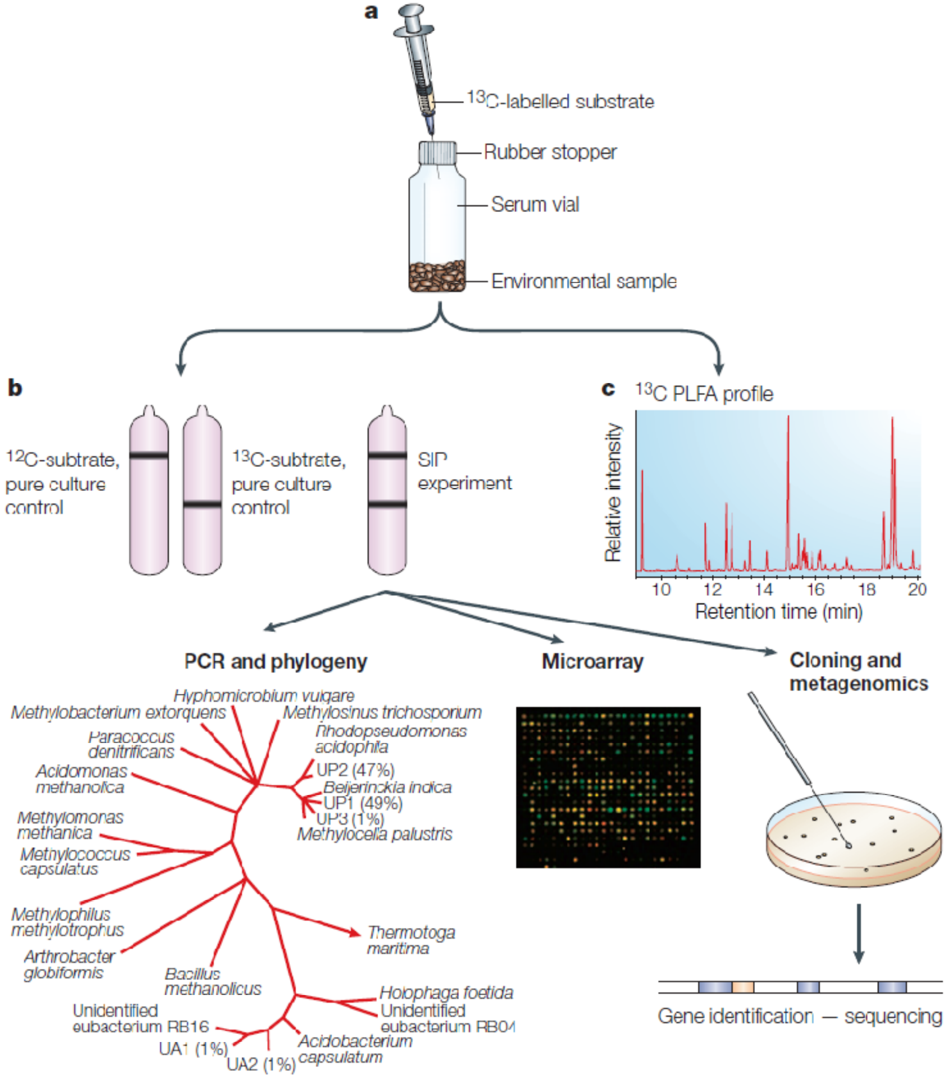


Fig. 7. The ^{13}C atom% excess in the four most highly enriched phospholipid fatty acids extracted from soil 4 and 8 days after pulse-labelling. Solid bars are for limed plots, grey bars are for control plots. Standard errors of the means are shown ($n = 3$). Data are recalculated from Treonis et al. (2004).

Stable isotope probing (From Dumont& Murrel, Nature, 2005)



DNA-based stable isotope probing (SIP) and ^{13}C-phospholipid fatty acids (PLFA) analyses. a | A ^{13}C-labelled substrate is added to an environmental sample, such as soil, water or plant material, either *in situ* in the field, or in a serum vial (as depicted). The sample is incubated so that the labelled carbon from the substrate can be incorporated into the biomass of the active microorganisms in the sample. **b** | Total DNA that has been purified from the incubated sample should represent those microorganisms that grew using the 13C-labelled substrate. This genomic DNA — enriched with the 13C isotope — can be separated from the community DNA (^{12}C-DNA) by CsCl gradient centrifugation. Phylogenetic analyses of sequence data produced by PCR amplification of the isolated 13C-labelled DNA using selected primers sets (chosen by the researcher based on their knowledge of probable community members) such as 16S rRNA, *pmoA* (particulate methane monooxygenase), *mmoX* (soluble methane monooxygenase), *cmuA* (chloromethane utilization) and *mxaF* (methanol dehydrogenase) can help to identify organisms that are active in the soil sample (phylogenetic tree reproduced, with permission, from Radajewski et al. 2000; UA1–2 and UP1–3 are cloned 16S rRNA gene PCR products). Microarrays can also be used to identify which of the amplified genes are the most numerous. **c** | PLFA can also be purified, and PLFA profiles can reveal which microorganisms incorporated the 13C isotope. Image reproduced, with permission, from Bull *et al.*, 2000

Linking organisms to functions:

Which microorganisms are able do degrade toluene (a common pollutant) in soils?

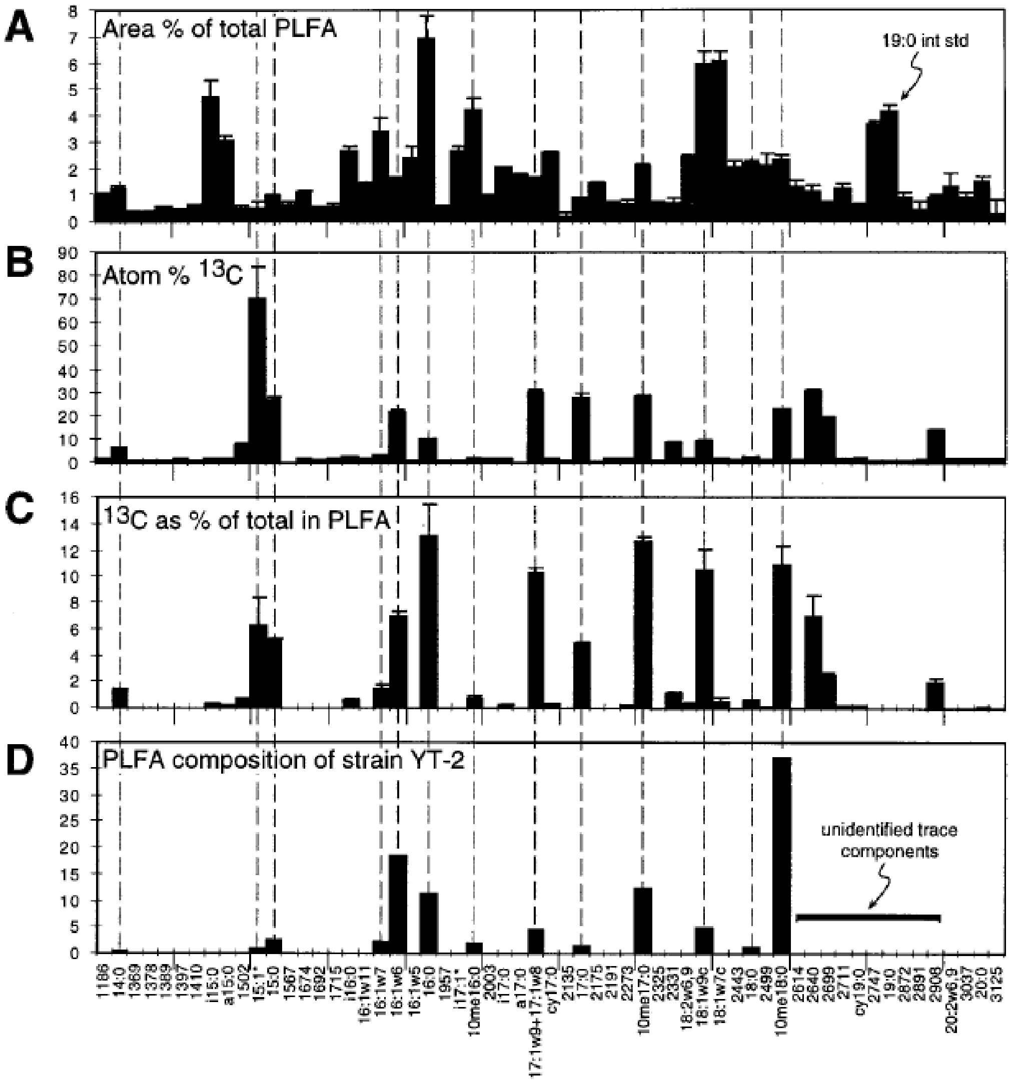


FIG. 2. Results of [^{13}C]toluene-labeling study in soil microcosms (±1 standard deviation; $n = 3$ microcosms). (A) PLFA area percentages. The initial toluene concentration (99 atom% ^{13}C in the toluene ring) was 30 mg liter of soil solution^{-1}. The microcosms were incubated for 119 h. int std, internal standard. (B) Atom percent ^{13}C values for individual PLFA shown in panel A. (C) Carbon-13 tracer content of each soil PLFA as a percentage of total ^{13}C enrichment in all PLFA. (D) PLFA composition of strain YT-2 grown on mineral medium plus toluene. The PLFA present in YT-2 grown on 0.1× tryptic soy broth were identical. Unidentified PLFA are denoted by their retention times in seconds.

Only the limited commercial availability of isotope labeled molecules suitable as microbial substrates constraints the use of this method!

Hanson et al., 1999

Linking organisms to functions:

Use of PLFA- SIP to study microbial community shifts under elevated CO_2

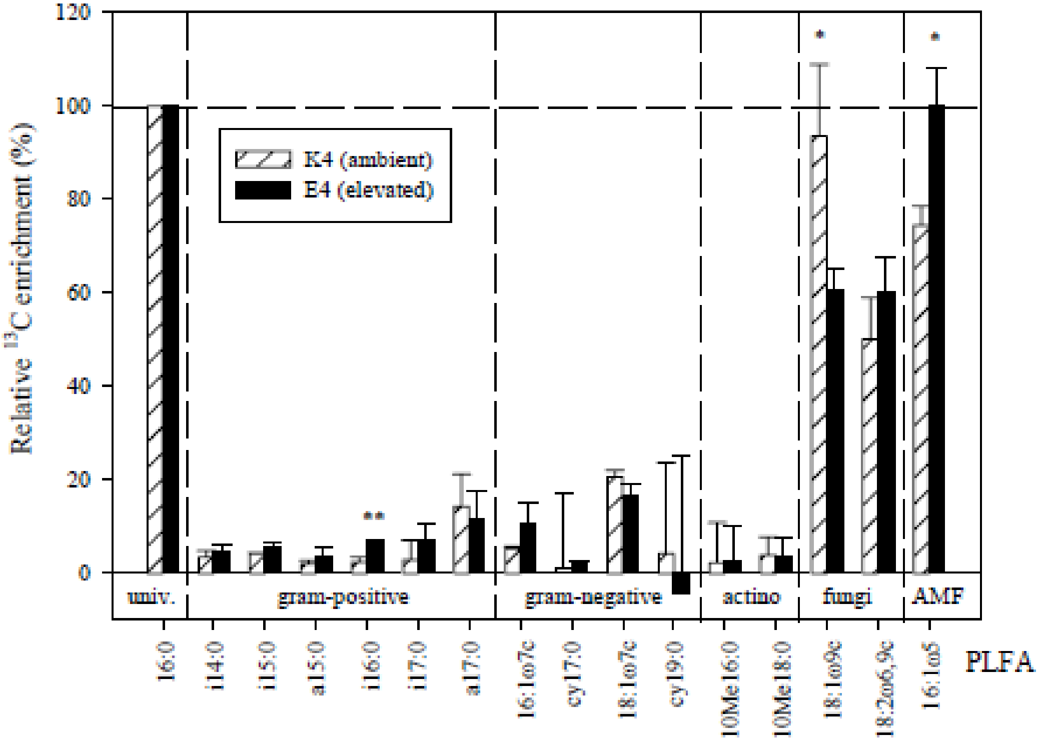


Fig. 4. Mean percentages of ^{13}C enrichment of individual biomarker PLFAs relative to the enrichment in ^{13}C of the universal 16:0 PLFA in 0–7.5 cm soil samples from plots K4 and E4 sampled after 10 h. Figure bars and error bars represent means and mean deviations for two subsamples from one composite soil sample of each pulse-labeled site (E4 and K4). * and ** indicate statistical differences at $P < 0.1$ and $P < 0.05$ respectively (based on two-sample *t*-test, $n=2$).

Giessen Free Air CO_2 Enrichment grassland

Denef et al., 2007

Stable isotope probing

Allows to move :

- From the lab to the environment
- From a discovery driven science to a hypothesis testing experiment
- From posing questions such as “who eats what?” to “Who eats what, when and where?”

If analyses are performed over time, it can be used also to reconstruct complex soil food-webs

Neufeld et al., 2007

Linking organisms to functions:

Fast advancing techniques!!

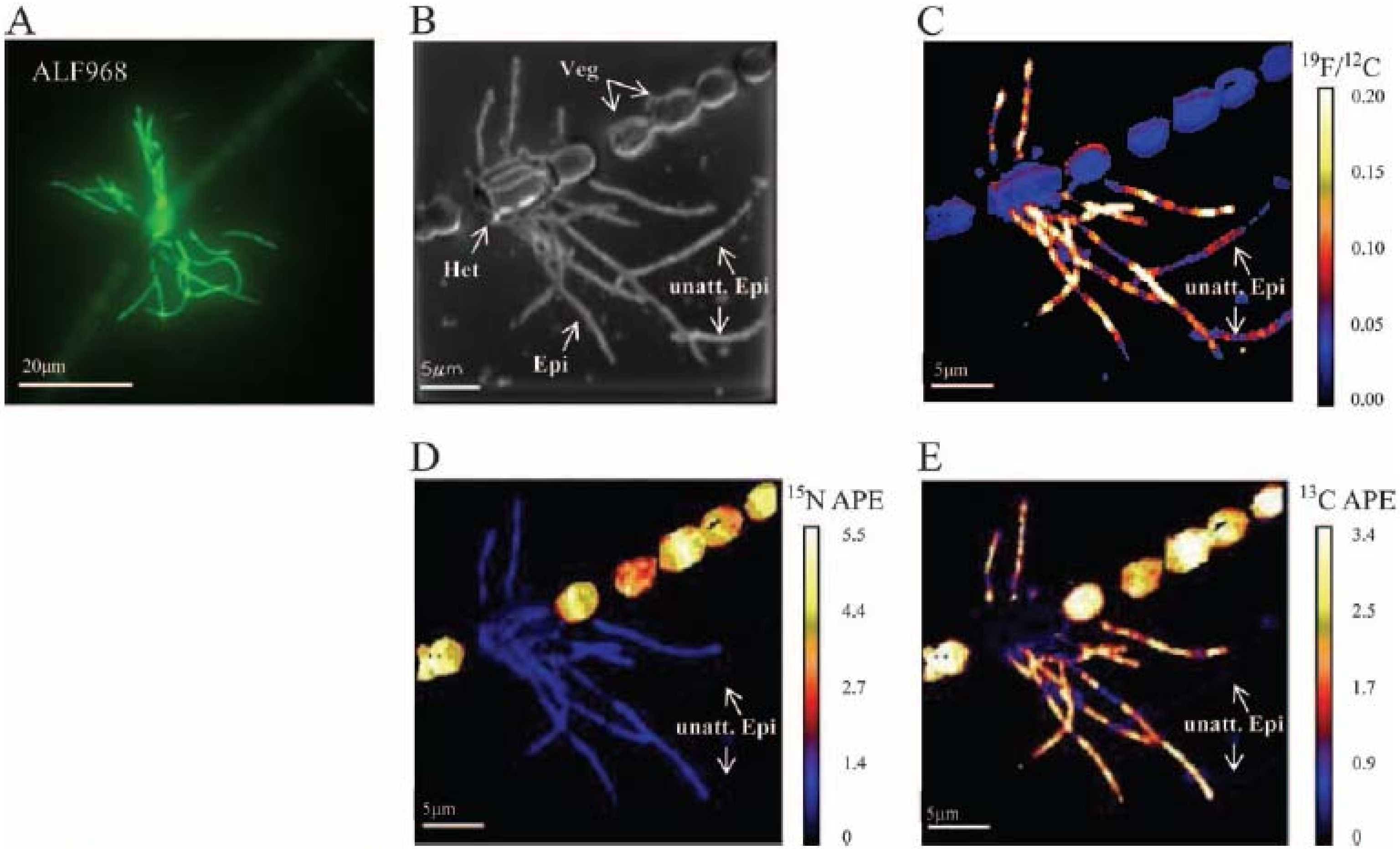


FIG. 2. Fluorescence and NanoSIMS images of a microbial consortium consisting of filamentous cyanobacteria (*Anabaena* sp. strain SSM-00) and alphaproteobacteria (*Rhizobium* sp. strain WH2K) attached to heterocysts. Images taken after a 24-h incubation with ^{13}C-bicarbonate and ^{15}N-dinitrogen. (A) Fluorescence image of the microbial consortium after EL-FISH with probe ALF968. (B) NanoSIMS secondary-electron image corresponding to panels C to E. (C) Localization of fluorine relative to carbon after EL-FISH with ALF968. (D) Distribution of ^{15}N-nitrogen enrichment. (E) Distribution of ^{13}C-carbon enrichment. Color bars indicate relative fluorine abundance (C) and isotope enrichment (D and E) in the image. Het, heterocyst; Veg, vegetative cell; Epi, epibiont; unatt Epi, Epibiont cells not attached to heterocysts.

Behrens et al., 2008

Tracing C to soil animal food webs

(From Pollierer et al 2007)

In a beech forest, on replicated plots, beech trees were continuously labeled with ^{13}C depleted CO$_2$. A litter transplant between labeled and control trees allowed for the establishment of three treatments: 1) only leaf litter labeled; 2) only root labeled; 3) leaf litter and root labeled. The analyses of δ^{13}C of mites helped reconstructing their feeding behavior

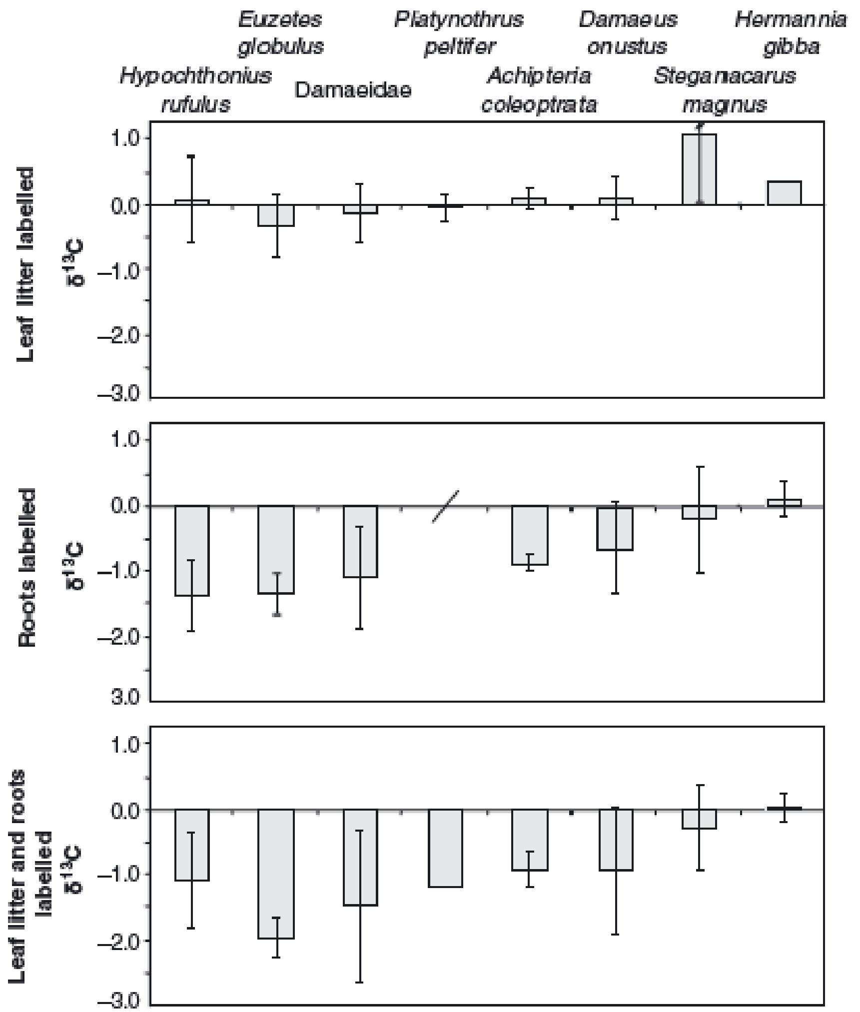


Figure 1 Differences in δ^{13}C signatures of oribatid mite taxa between the control and treatments with leaf litter labelled, roots labelled, and leaf litter and roots labelled. Data are presented as mean ± SD (bars without SD are single measurements).

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