Determination of compound-specific isotope ratios

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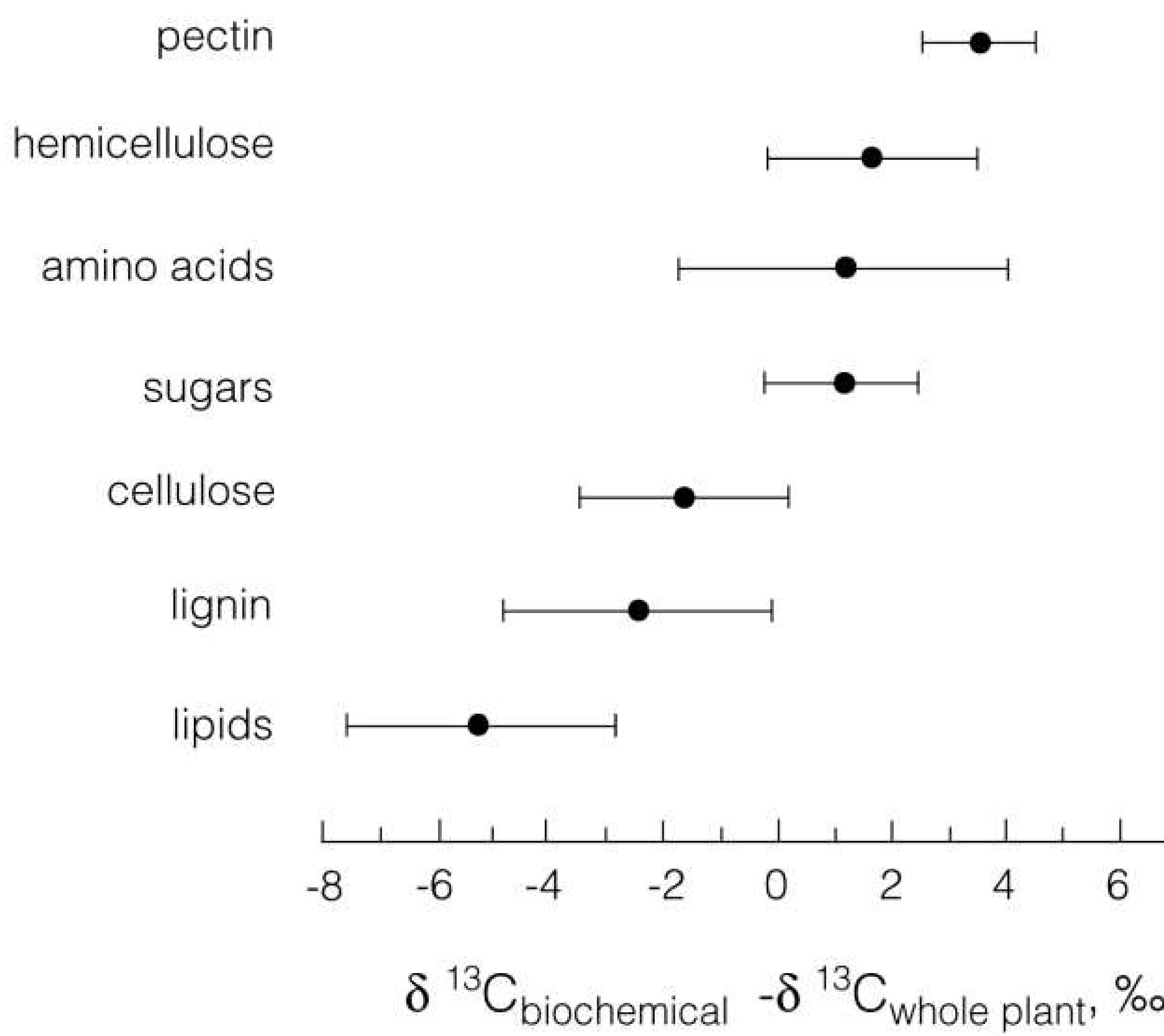
Molecular composition of cells

Table 1. Overall macromolecular composition of an average *E. coli* B/r cell[a]

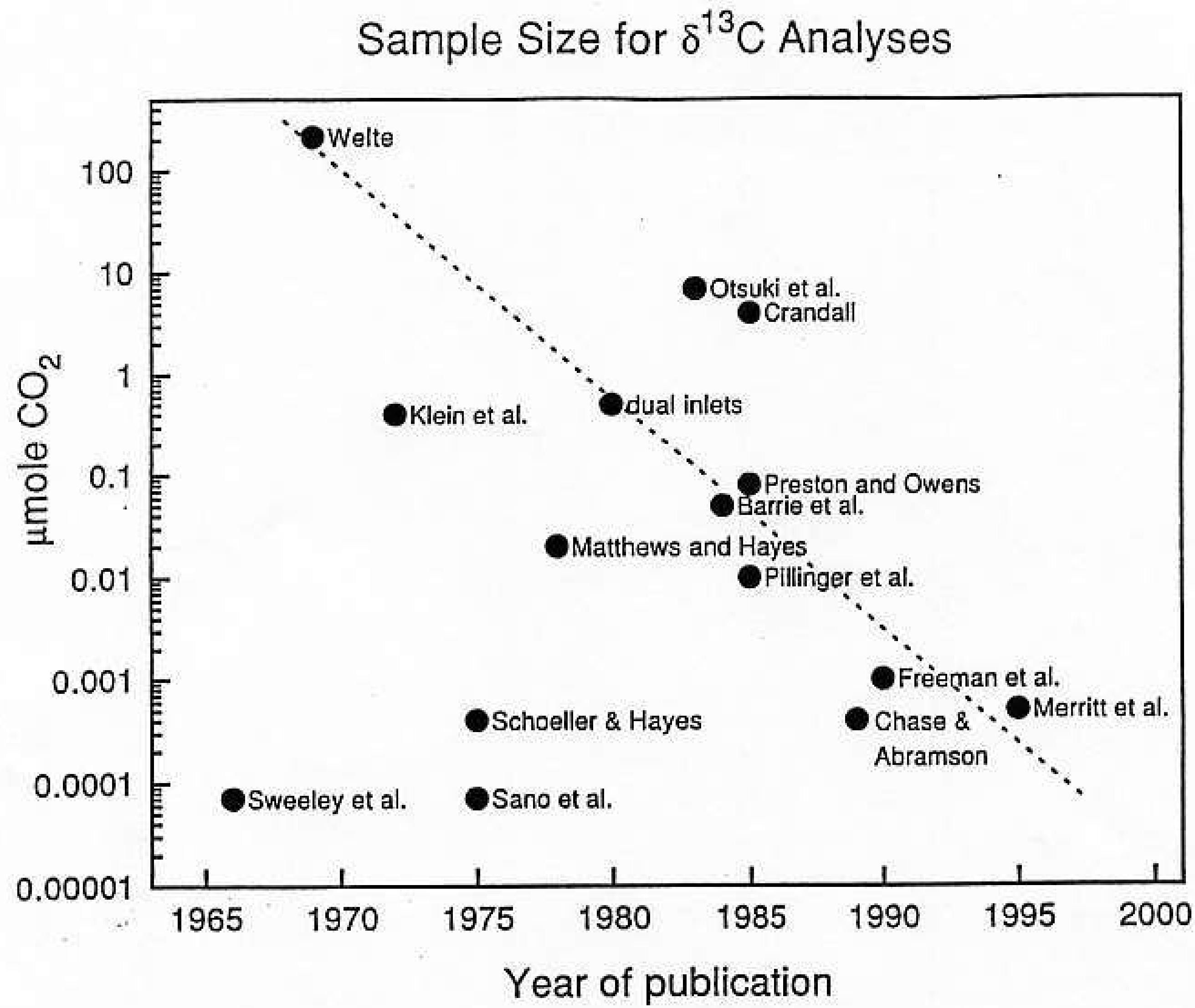
Macromolecule	Percentage of total dry weight	Weight per cell (10^{15} × weight, grams)		Molecular weight	Number of molecules per cell	Different kinds of molecules
Protein	55.0	155.0		4.0×10^4	2,360,000	1,050
RNA	20.5	59.0				
23S rRNA			31.0	1.0×10^6	18,700	1
16S rRNA			16.0	5.0×10^5	18,700	1
5S rRNA			1.0	3.9×10^4	18,700	1
transfer			8.6	2.5×10^4	205,000	60
messenger			2.4	1.0×10^6	1,380	400
DNA	3.1	9.0		2.5×10^9	2.13	1
Lipid	9.1	26.0		705	22,000,000	4[b]
Lipopolysaccharide	3.4	10.0		4346	1,200,000	1
Murein	2.5	7.0		$(904)_n$	1	1
Glycogen	2.5	7.0		1.0×10^6	4,360	1
Total macromolecules	96.1	273.0				
Soluble pool	2.9	8.0				
building blocks			7.0			
metabolites, vitamins			1.0			
Inorganic ions	1.0	3.0				
Total dry weight	100.0	284.0				
Total dry weight/cell		2.8×10^{-13}g				
Water (at 70% of cell)		6.7×10^{-13}g				
Total weight of one cell		9.5×10^{-13}g				

[a] In balanced growth at 37°C in glucose minimal medium, mass doubling time, *g*, of 40 minutes. The data are assembled from Dennis and Bremer (1974), Maaløe (1979), F. C. Neidhardt (unpublished), Roberts et al. (1955), and Umbarger (1977).
[b] There are four classes of phospholipids, each of which exists in many varieties as a result of variable fatty acyl residues.

Intermolecular variations in ^{13}C composition

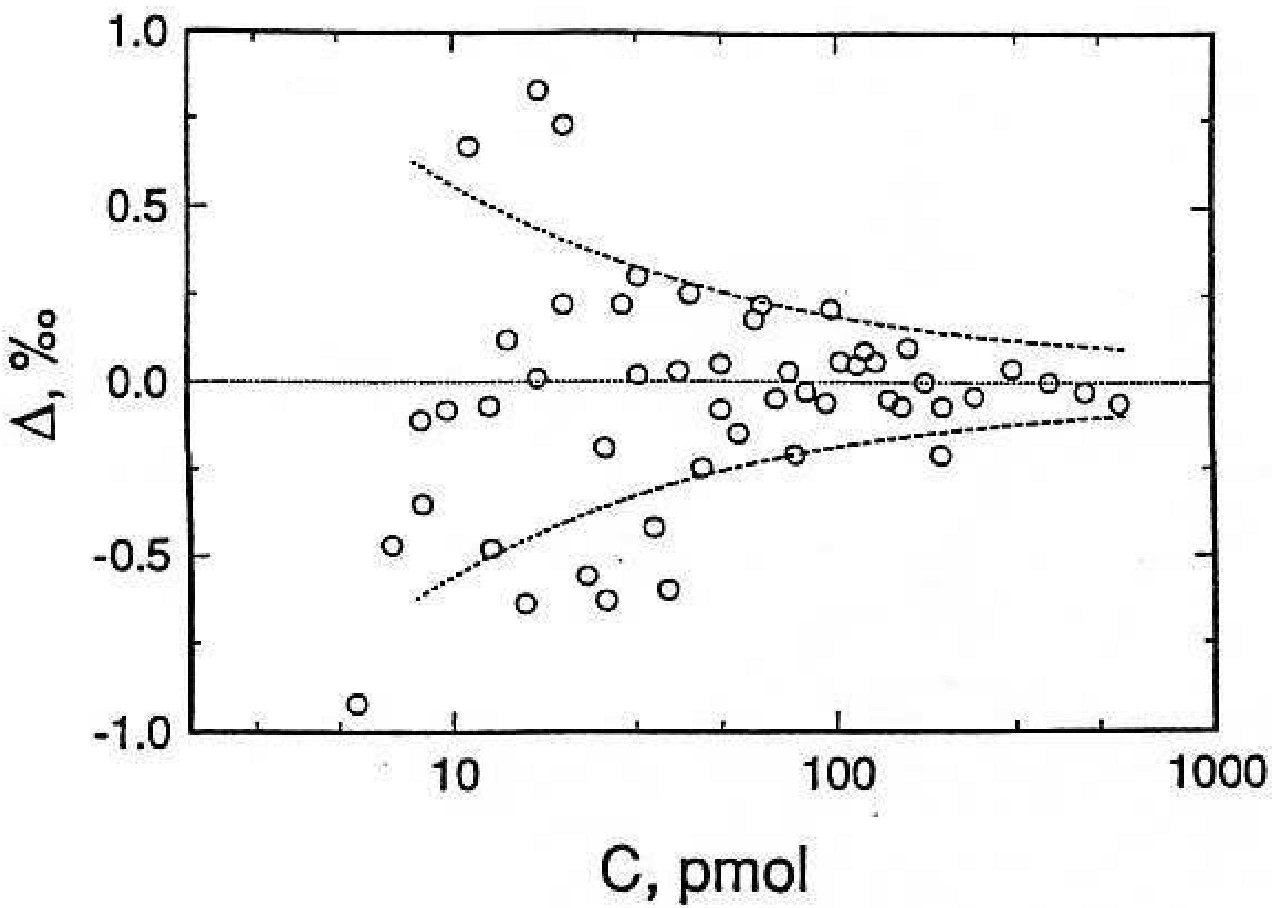


Boutton (1996)



Sample Size for δ13C Analyses
μmole CO2
100
10
1
0.1
0.01
0.001
0.0001
0.00001
Welte
Otsuki et al.
Crandall
Klein et al.
dual inlets
Preston and Owens
Barrie et al.
Matthews and Hayes
Pillinger et al.
Freeman et al.
Merritt et al.
Schoeller & Hayes
Chase &
Abramson
Sweeley et al.
Sano et al.
1965
1970
1975
1980
1985
1990
1995
2000
Year of publication

Noise limits

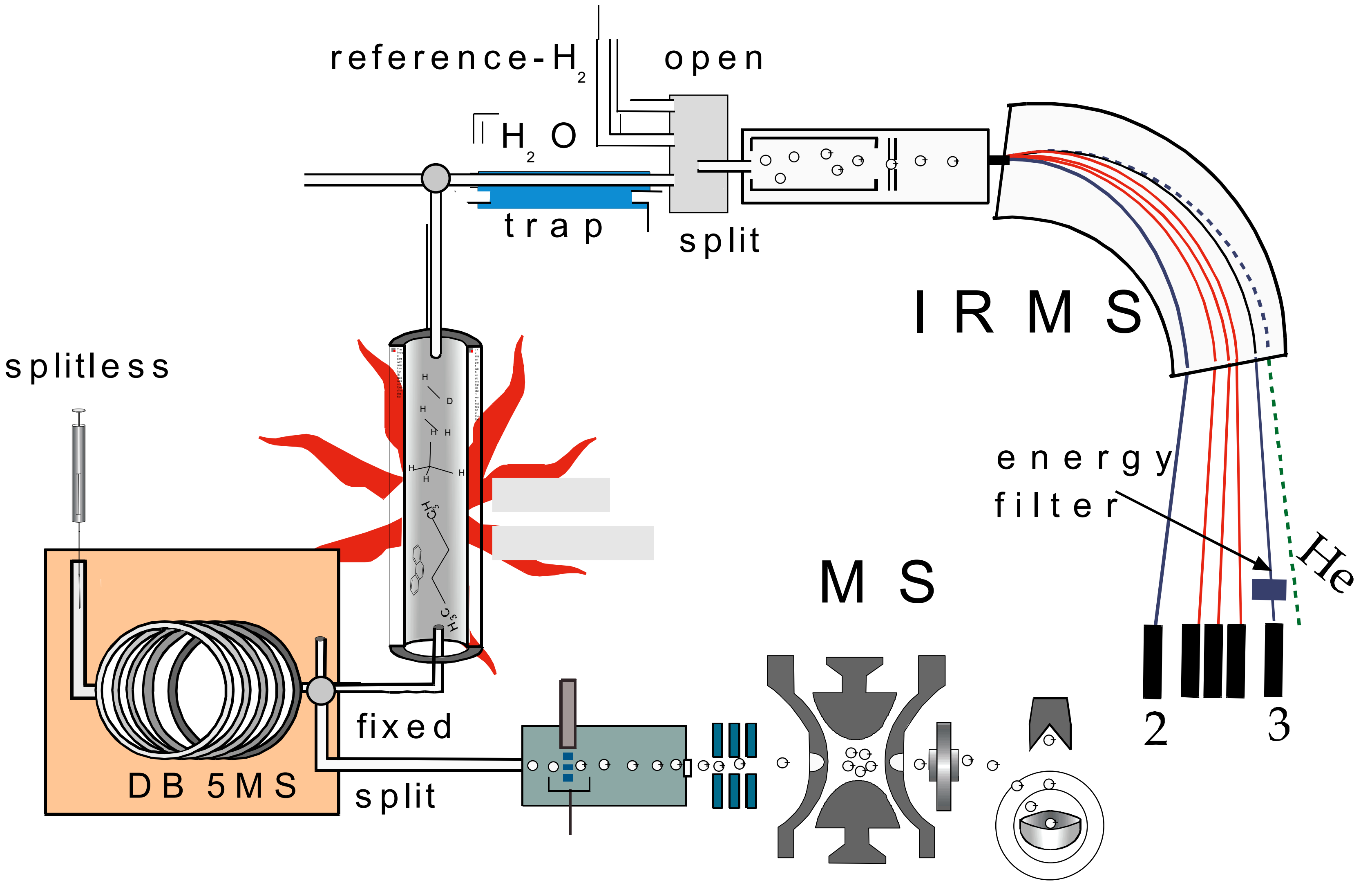


Merritt,93

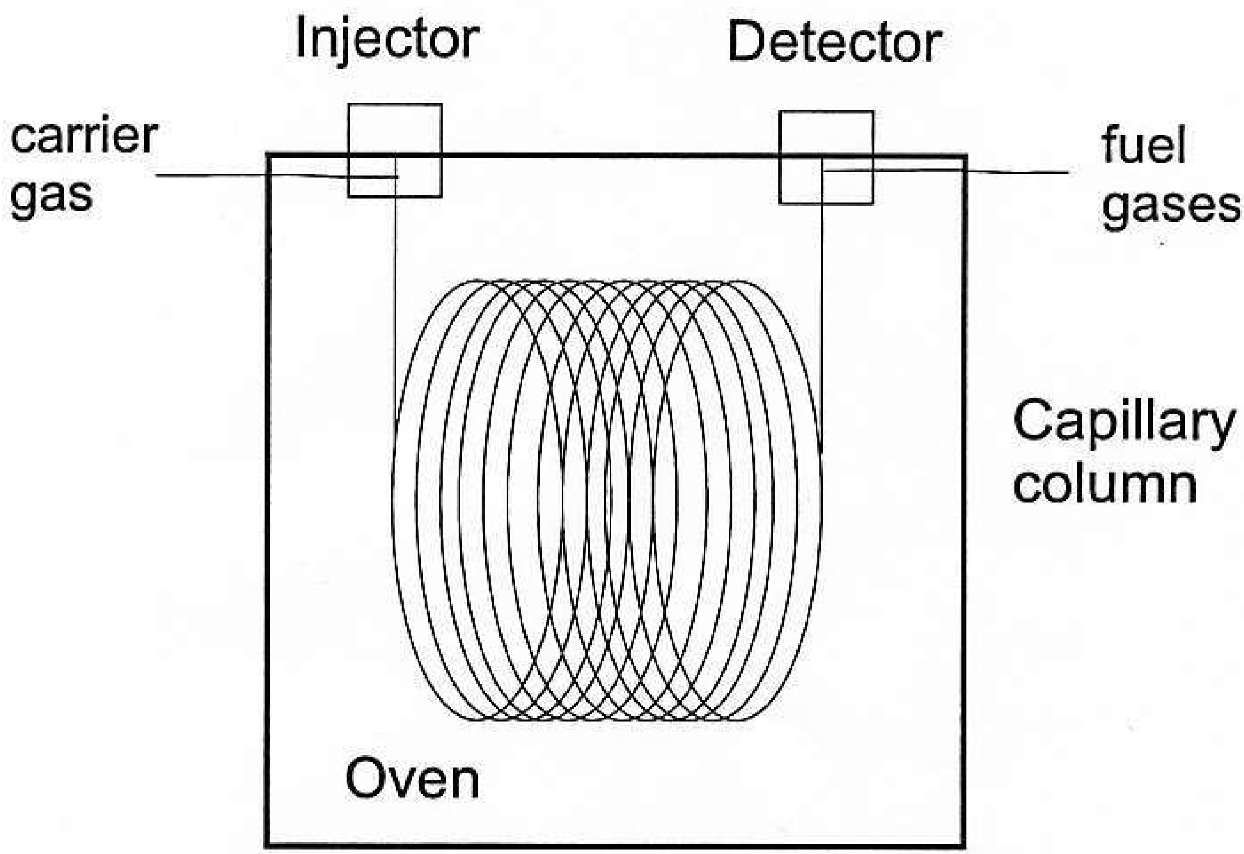
GC-IRMS for C, N and H

- Gas chromatographic separation
- Conversion of compounds
- Water removal
- Coupling to IRMS

GC/MS-IRMS


reference-H2
open
H2O
trap
split
IRMS
splitless
energy filter
He
MS
fixed
split
DB 5MS
2
3

The Gas Chromatograph System



Column- the heart of separation

Säulentypen

- Gepackte Säule (innerer Durchmesser 1-4 mm, Länge 1-5 m)
- Kapillarsäule (*porous-layer open tubular*, PLOT; *support-coated open tubular*, SCOT; *wall-coated open tubular*, WCOT; innerer Durchmesser 0.15-0.53mm, Länge 10-100 m)

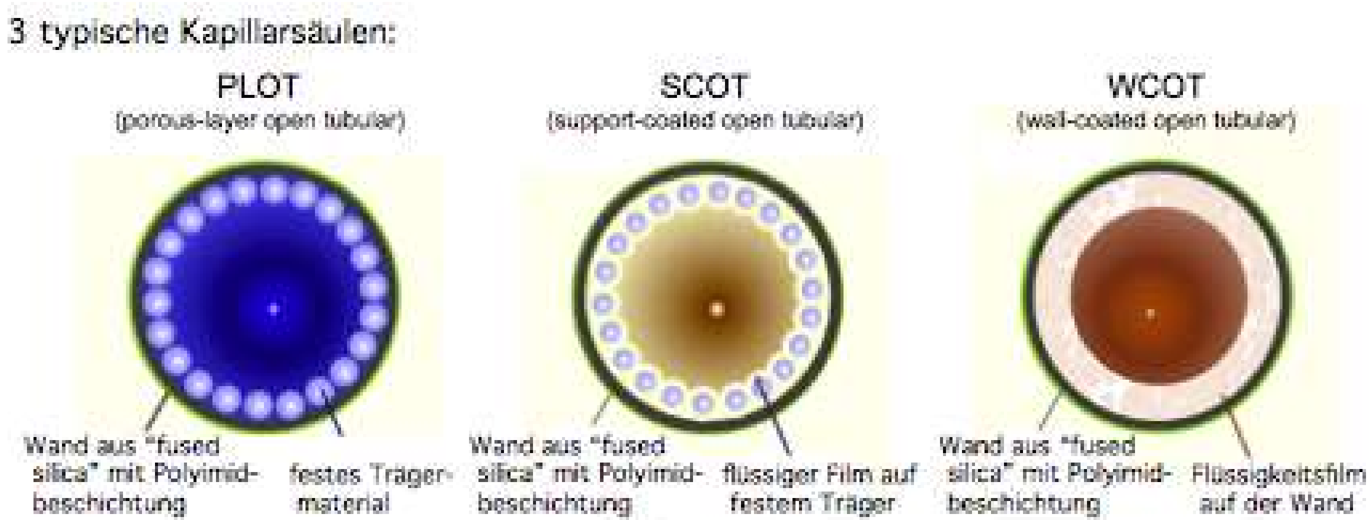
Gepackte Säulen

Kapillarsäule

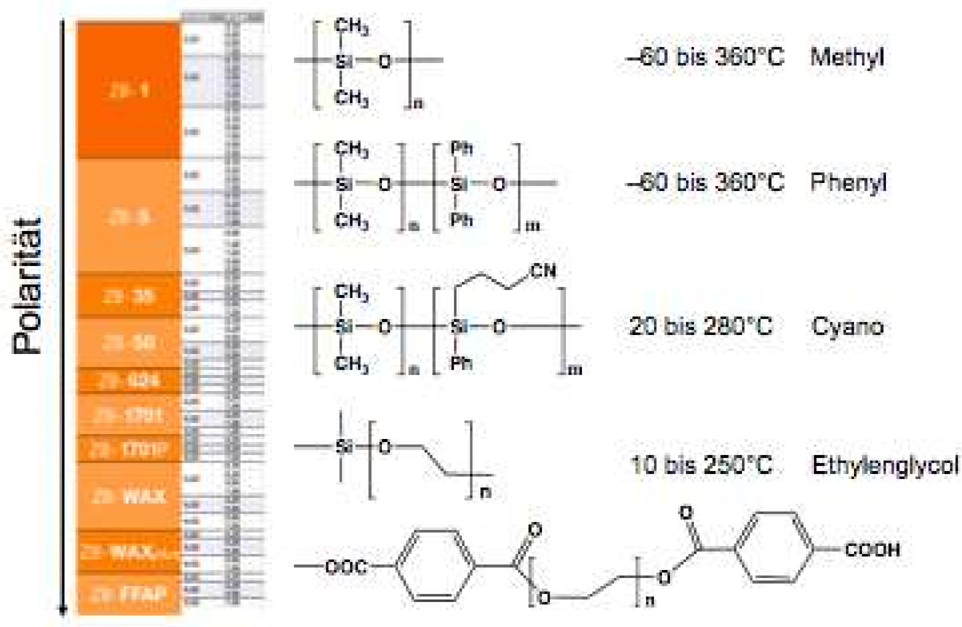
Vergleich zwischen Kapillarsäule und gepackter Säule:

	Gepackte Säule	Kapillarsäule
Länge (m)	1 – 5	10 – 100
Innendurchmesser (mm)	1 – 4	0.1 – 0.5
Theoretische Bodenzahl per Meter	500 – 1000	2000 – 4000
Probemengen	ng – mg	< 10 ng

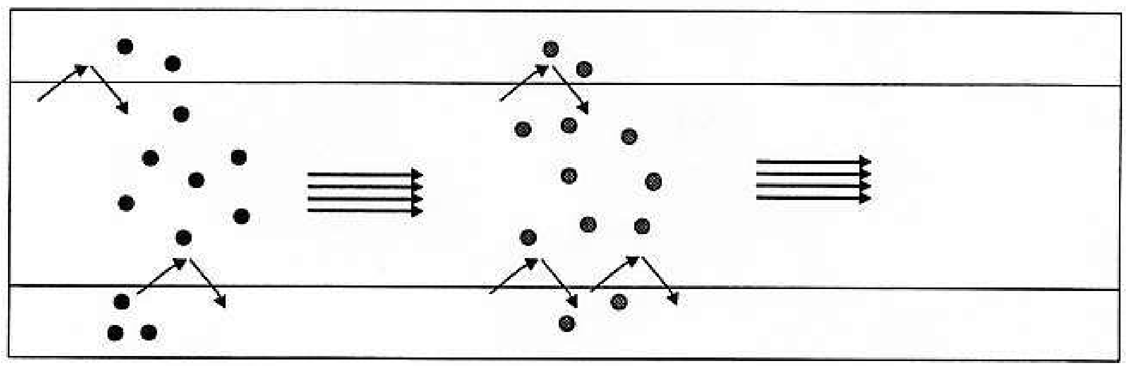
Säulentypen



Stationäre Phasen



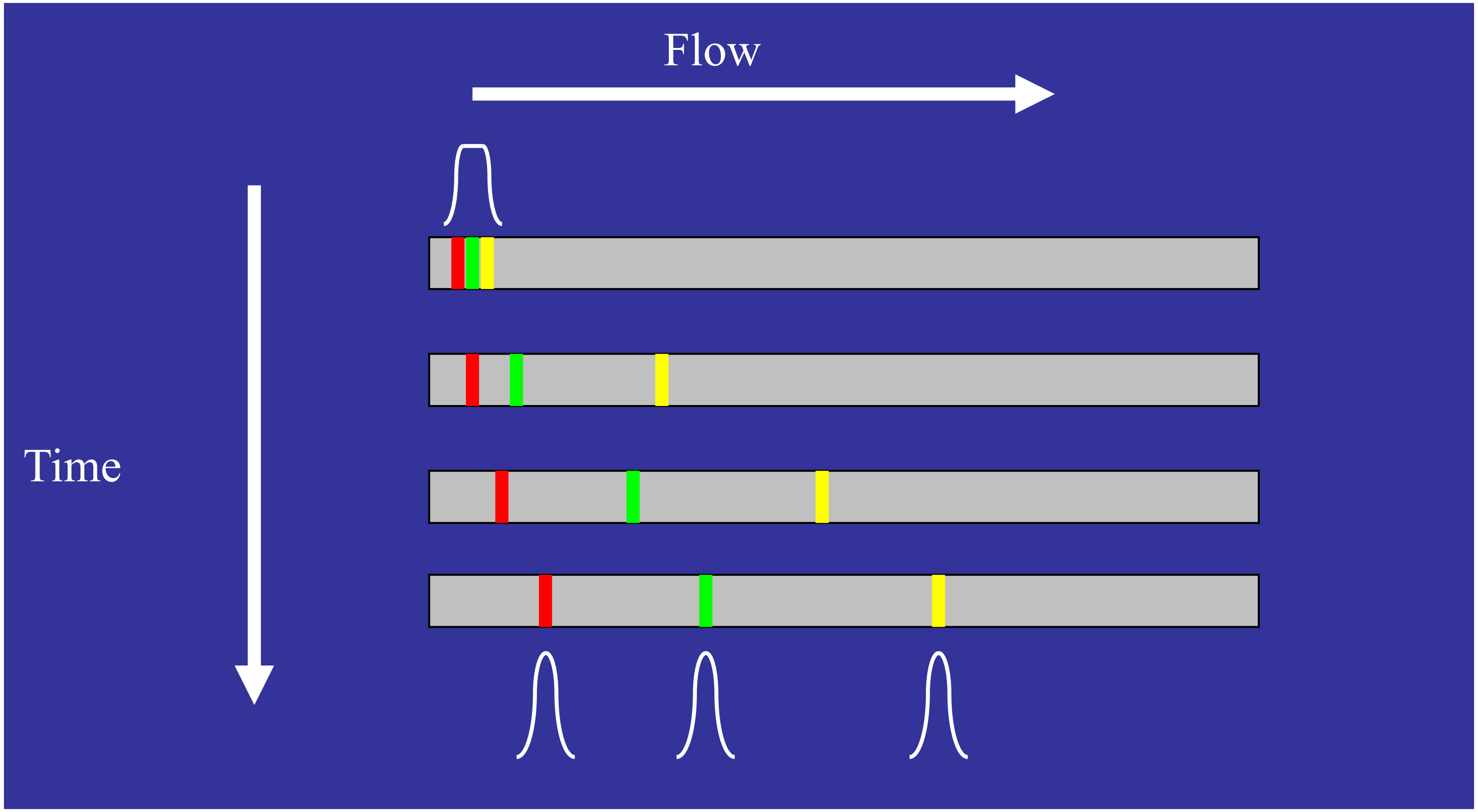
Capillary Column Chromatography



Separation power depends on:

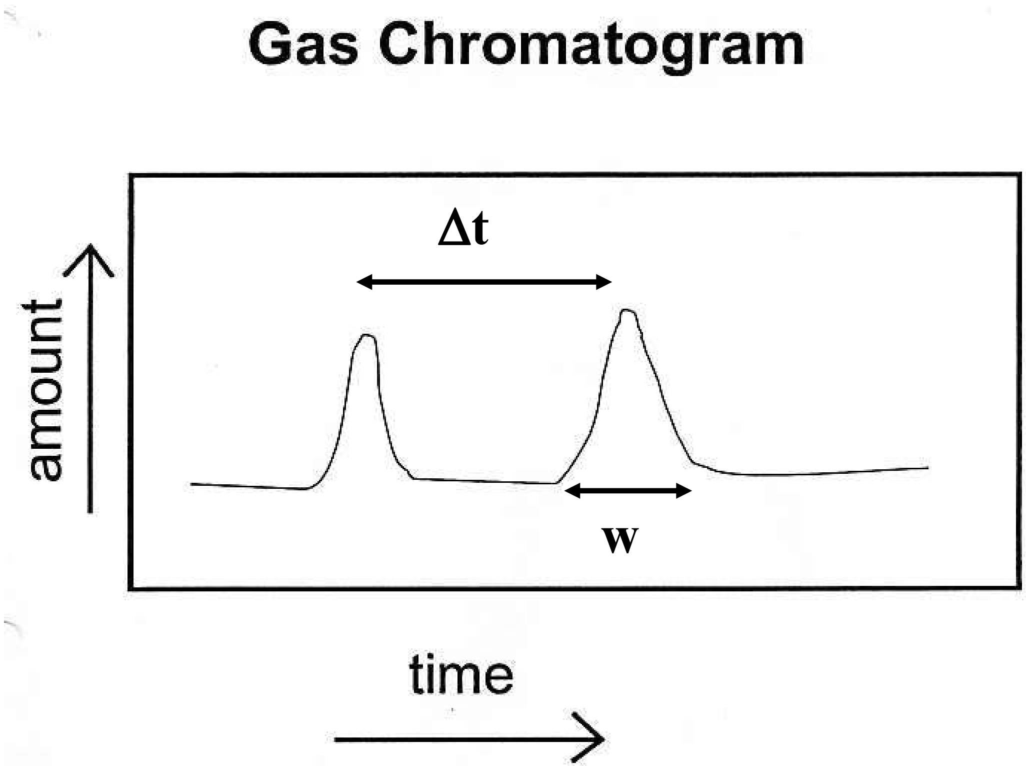
chemistry of compounds
chemistry of bonded phase
temperature and flow programs

Chromatographic separation



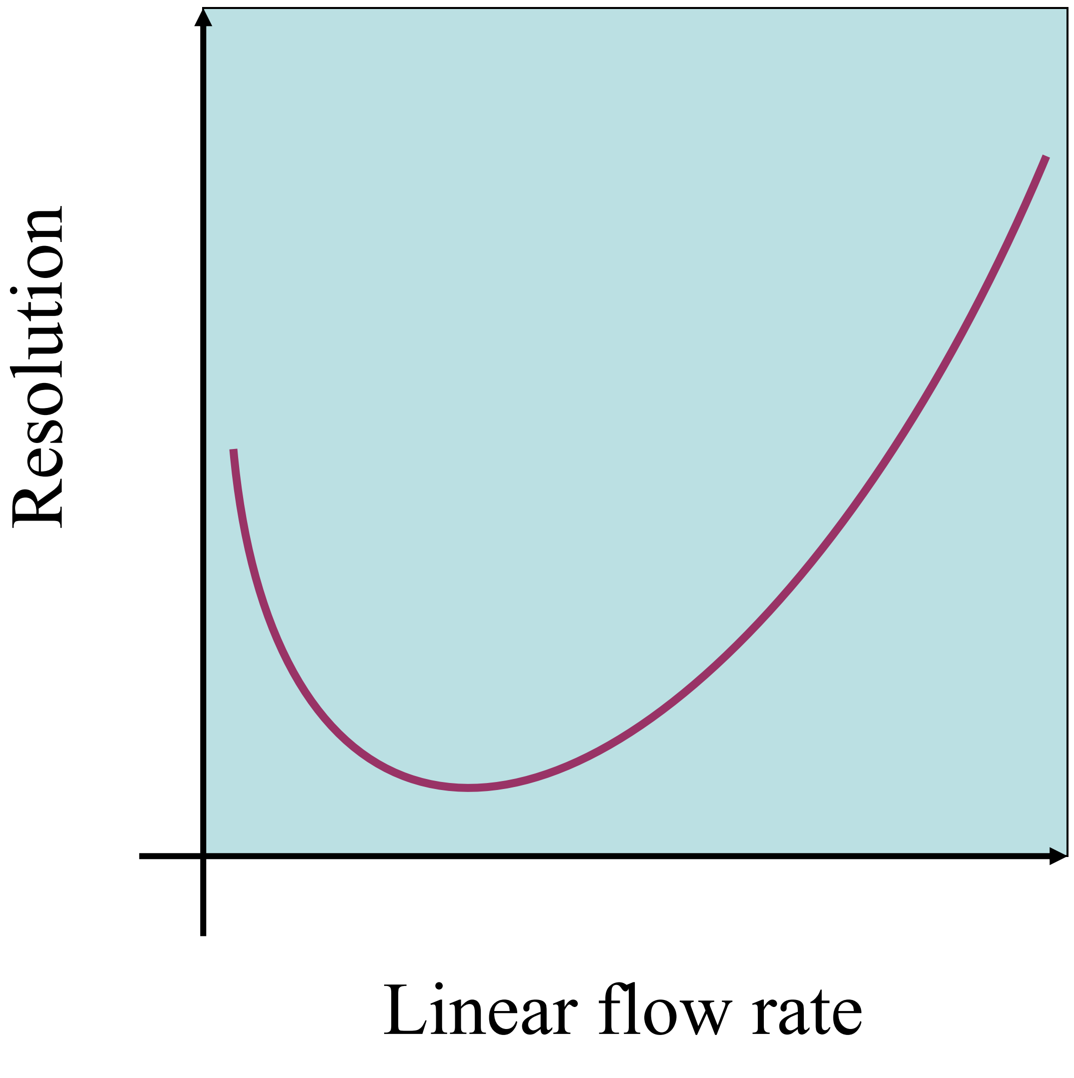
Multiple distribution of compounds in non mixing phases

Peak resolution

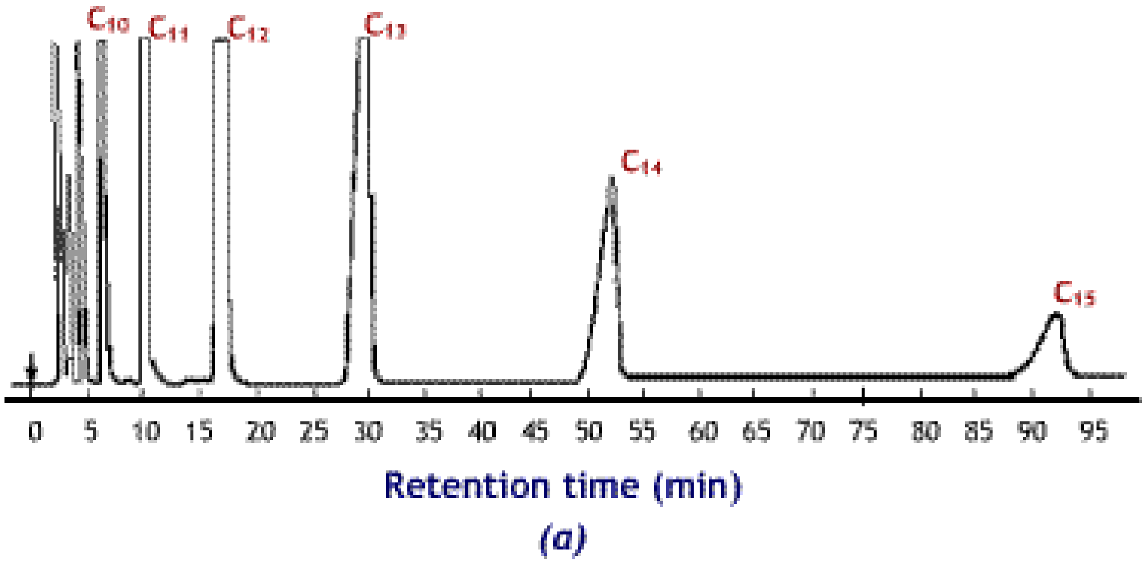


Resolution = Δt / w

Column Flow

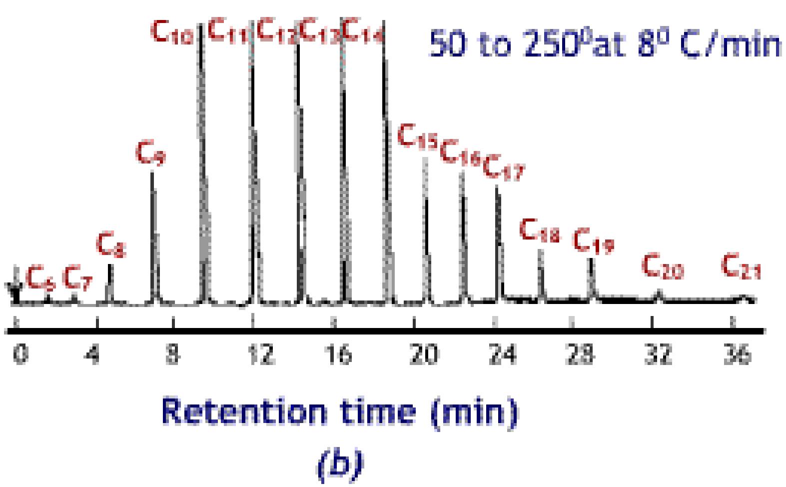


Temperature



Isothermal

(constant temperature)



Temperature Programmed

(increasing temperature)

http://www.uga.edu/srel/AACES/GCtutorial

Gas flow in a capillary tube:

Poiseulle Equation

$$\text{flow rate (mL/min)} = \frac{k\, r^4 \Delta P}{l\eta}$$

k = constant
r = capillary radius (cm)
ΔP = pressure gradient (dynes/cm)
l = capillary length (cm)
η = viscosity (dyne-sec/cm^2)--*increases with temperature*

Gas flow in a capillary tube:

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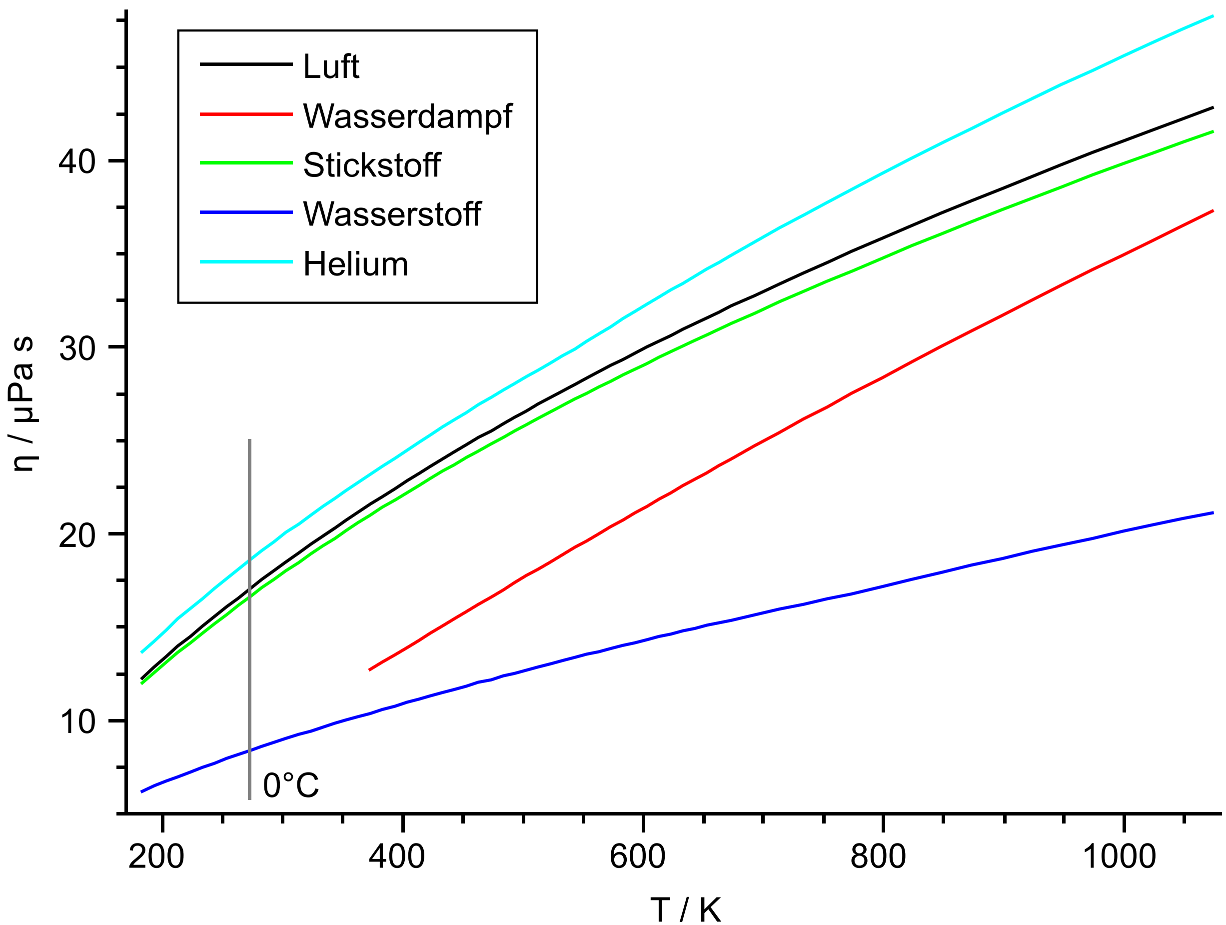
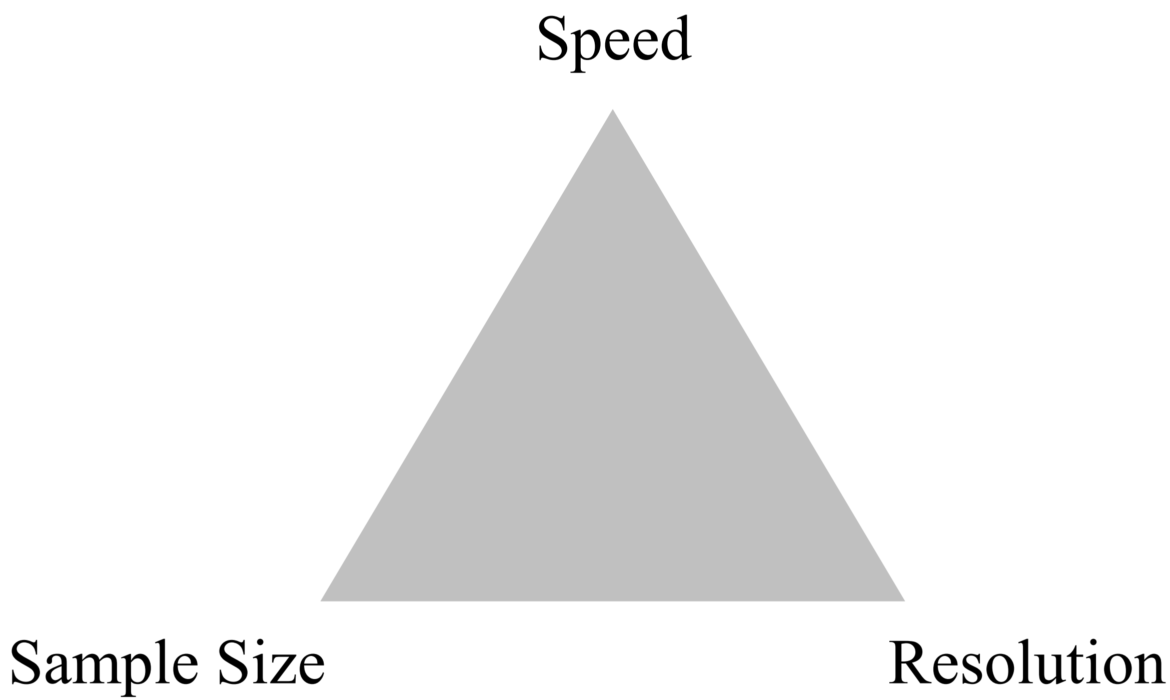
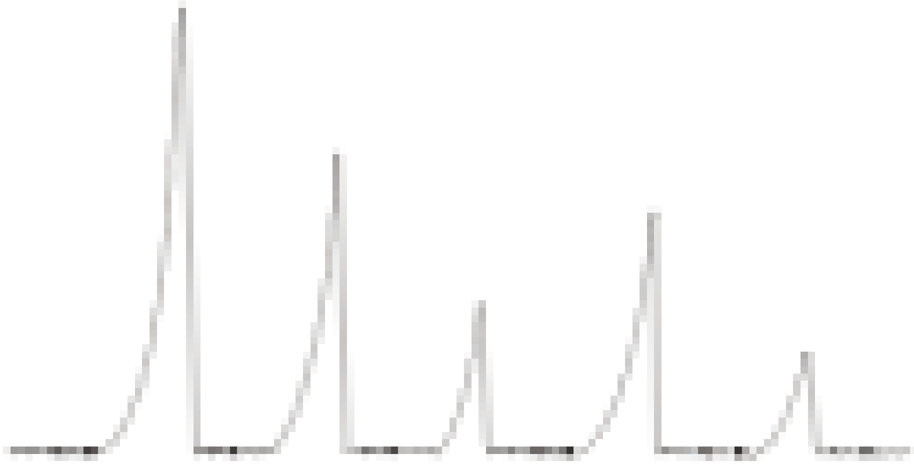
k = constant
r = capillary radius (cm)
ΔP = pressure gradient (dynes/cm)
l = capillary length (cm)
η = viscosity (dyne-sec/cm^2)--*increases with temperature* **= flow rate drops**


Luft
Wasserdampf
Stickstoff
Wasserstoff
Helium
0°C
η / µPa s
10
20
30
40
200
400
600
800
1000
T / K

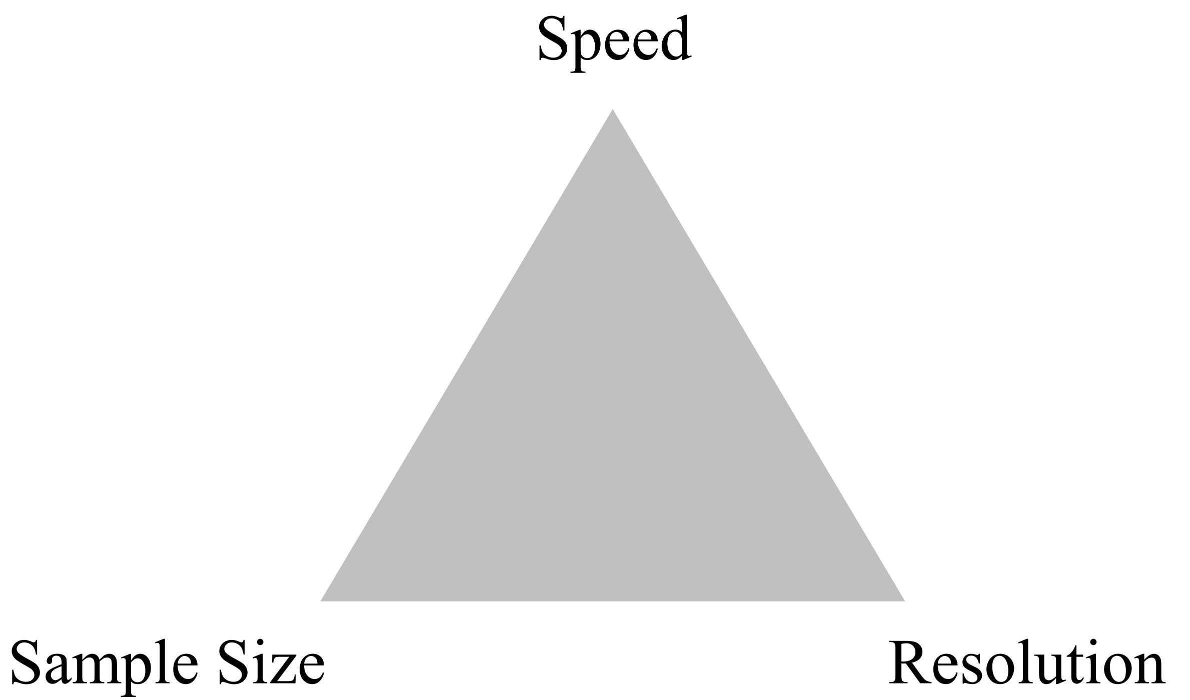
Separation optimization



Säulenüberladung



Separation optimization



Conversion

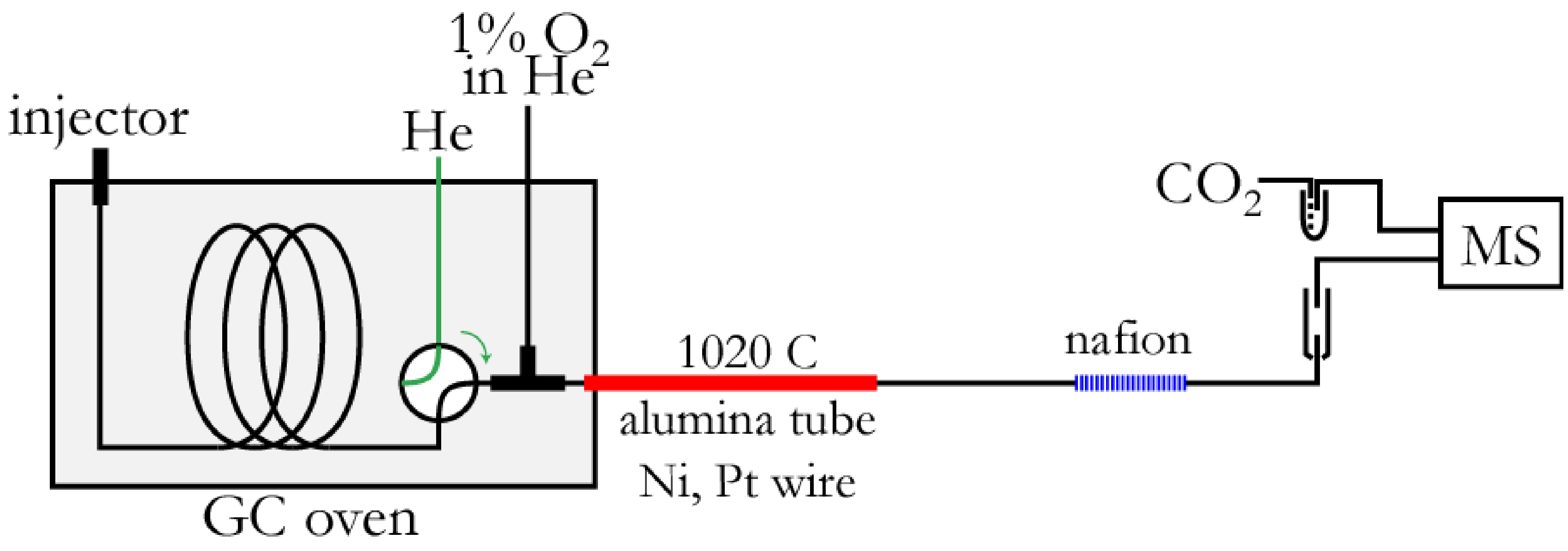
$$H_xC_yO_z$$

$$\downarrow \Delta T,\ O_2$$

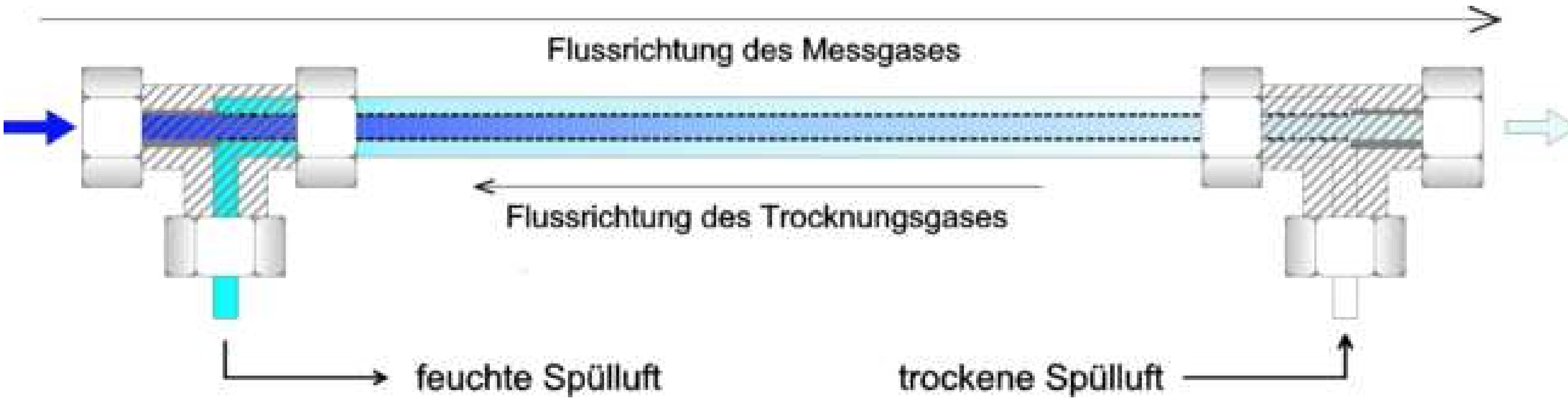
$$y\ CO_2 + x/2\ H_2O$$

$^{13}C/^{12}C$ irm-GCMS

At 1000 C w/O_2:

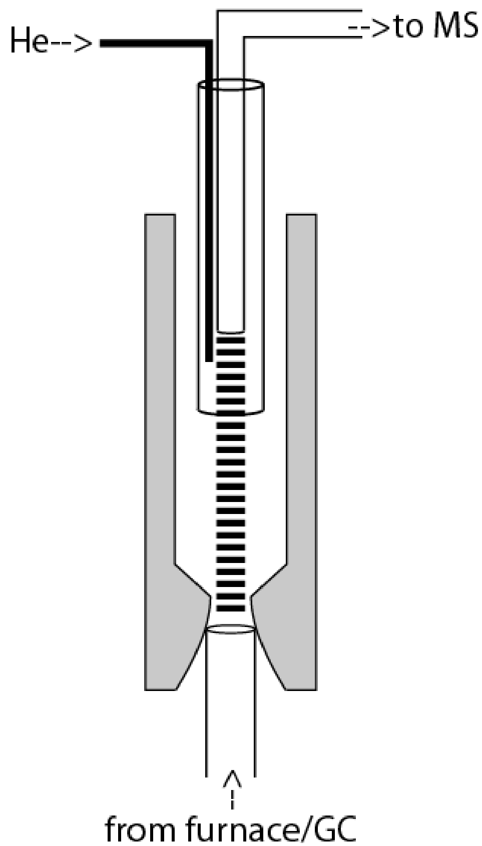


Water removal

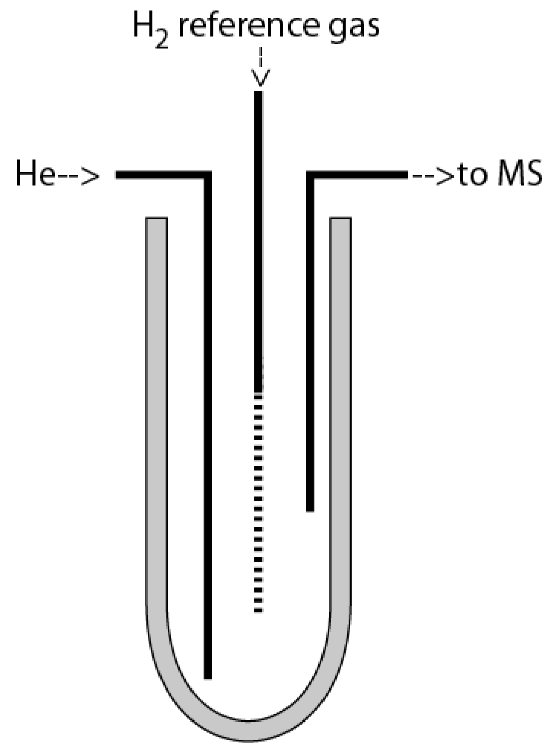


Open Splits

Sample split



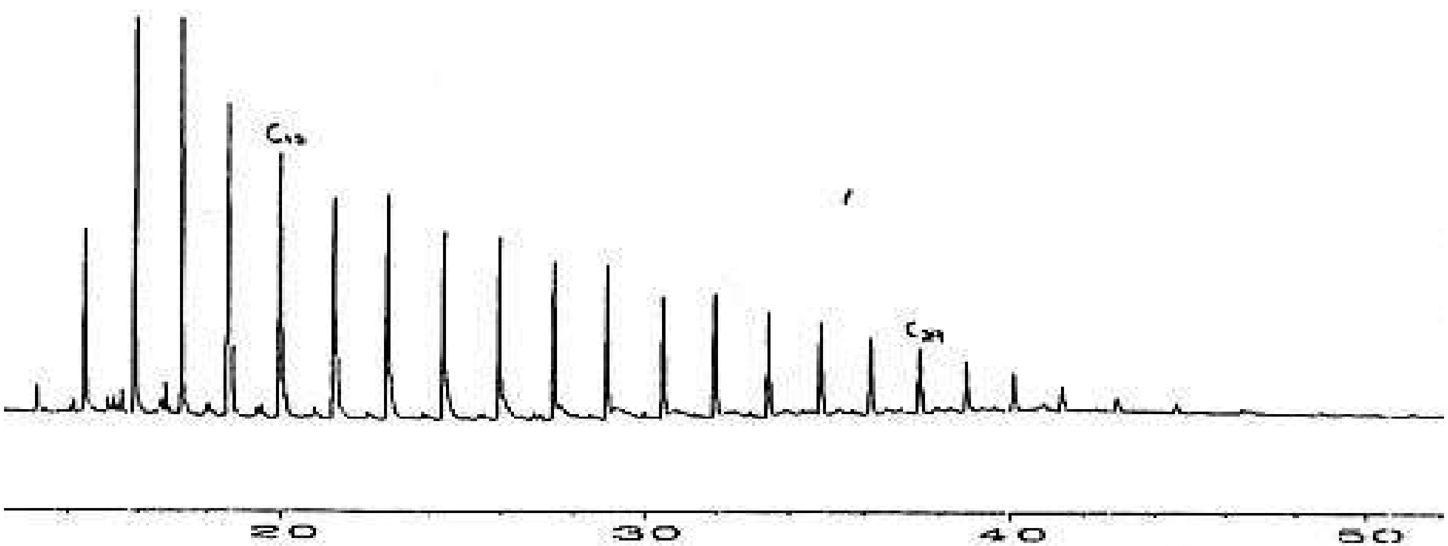
Reference split



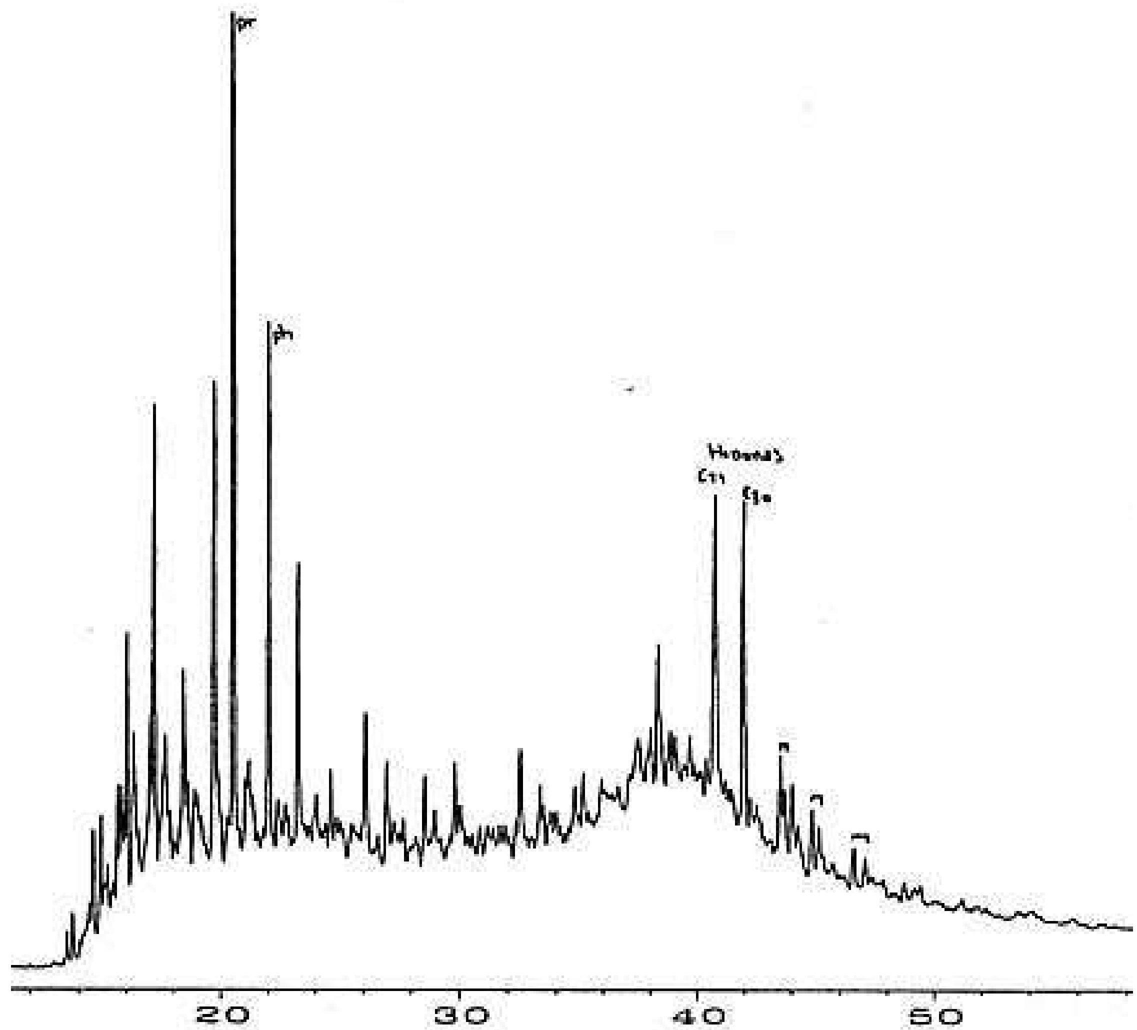
Compound-Specific ^{13}C Analyses

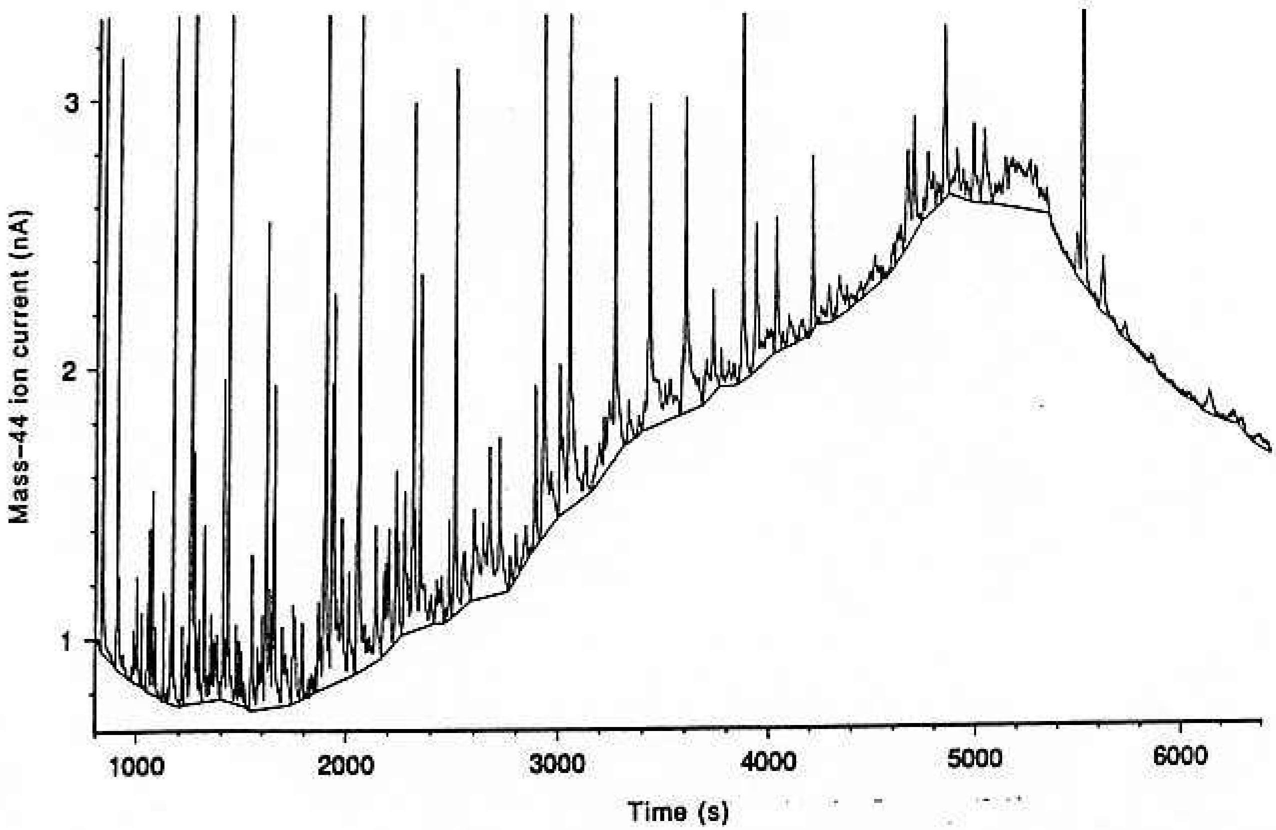
- Good chromatographic separation
- internal and external standards
- N can interfere with C analysis
  (N2O, NO2 have m/e 44, 46)
- Correction for derivatization of functional groups (methyl esters, TMS ethers, etc.)

A “typical” chromatogram

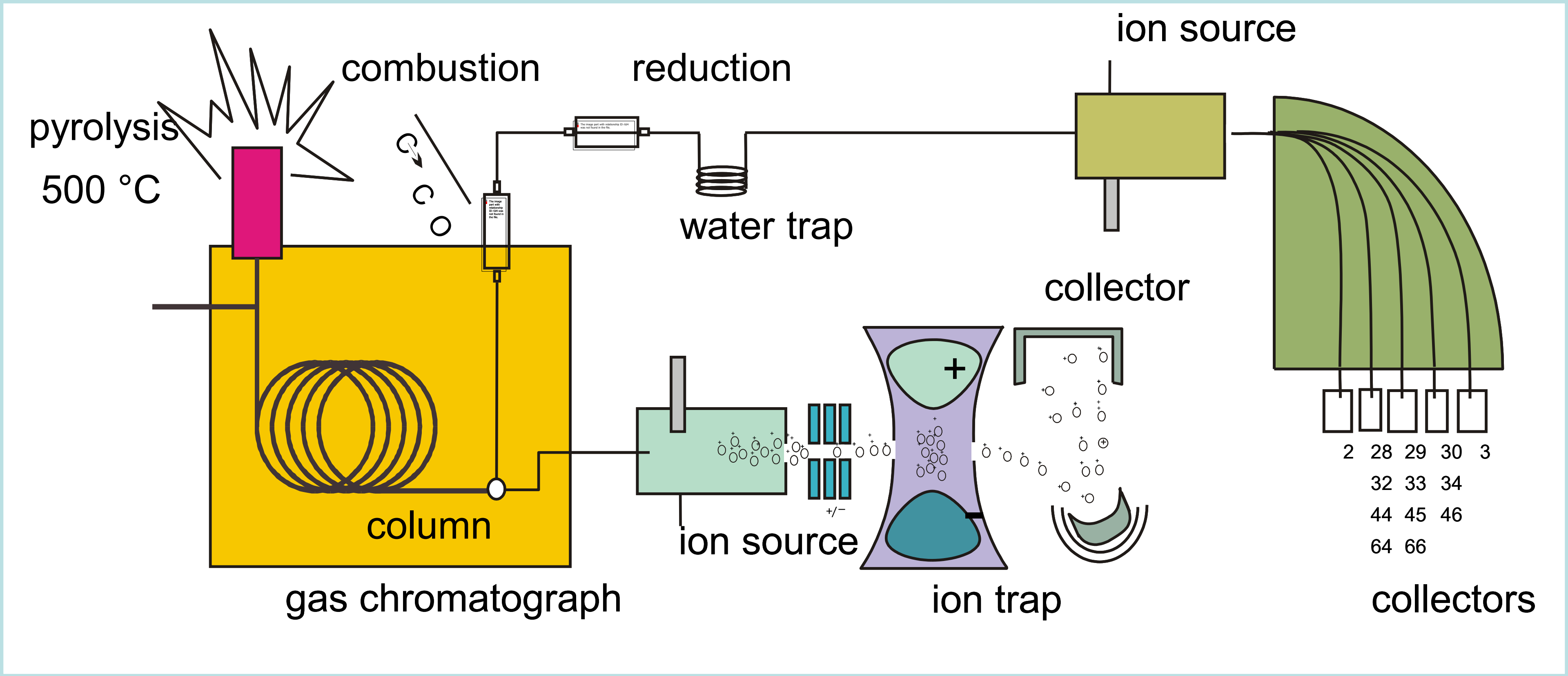


A real chromatogram





"Dynamic Background"



Precursor	Pyrolysis Products
Carbohydrate	Acetic Acid, Furanes
Protein	Nitriles, Pyrroles
Lignin	Phenols

after Gleixner et al., 1999

Nitrogen isotopes in GC-IRMS

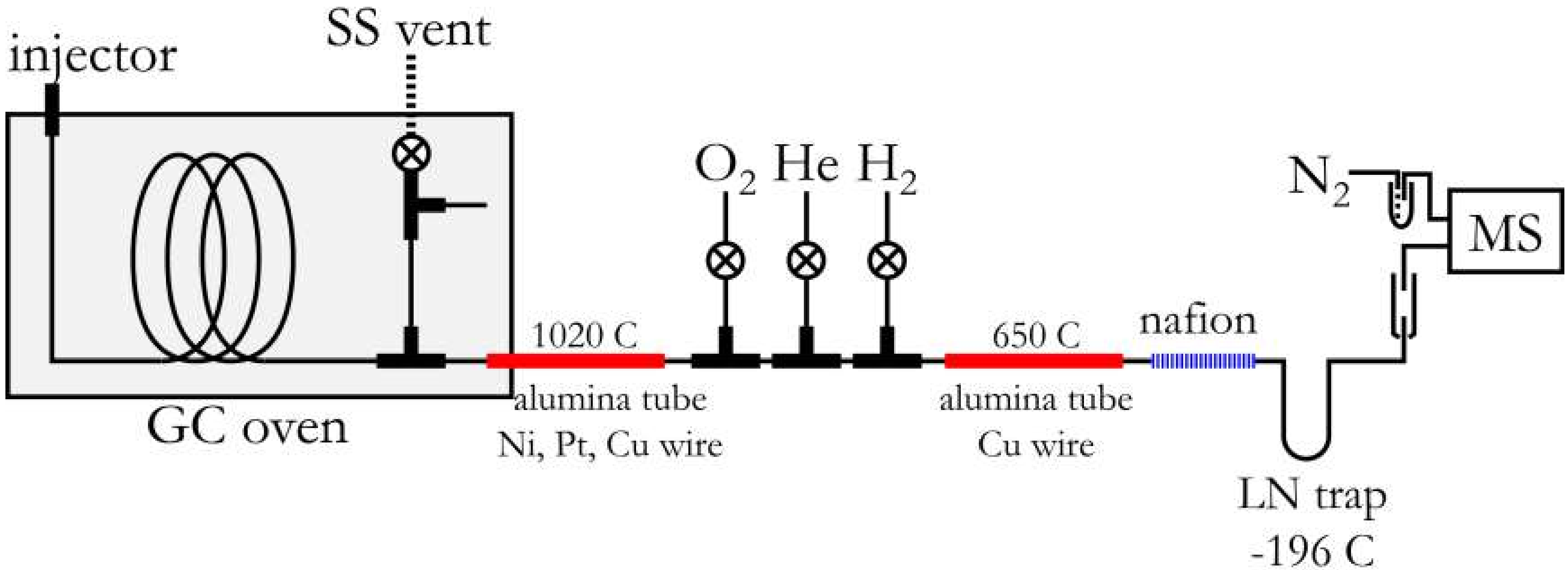
Oxidation at 1000° C:

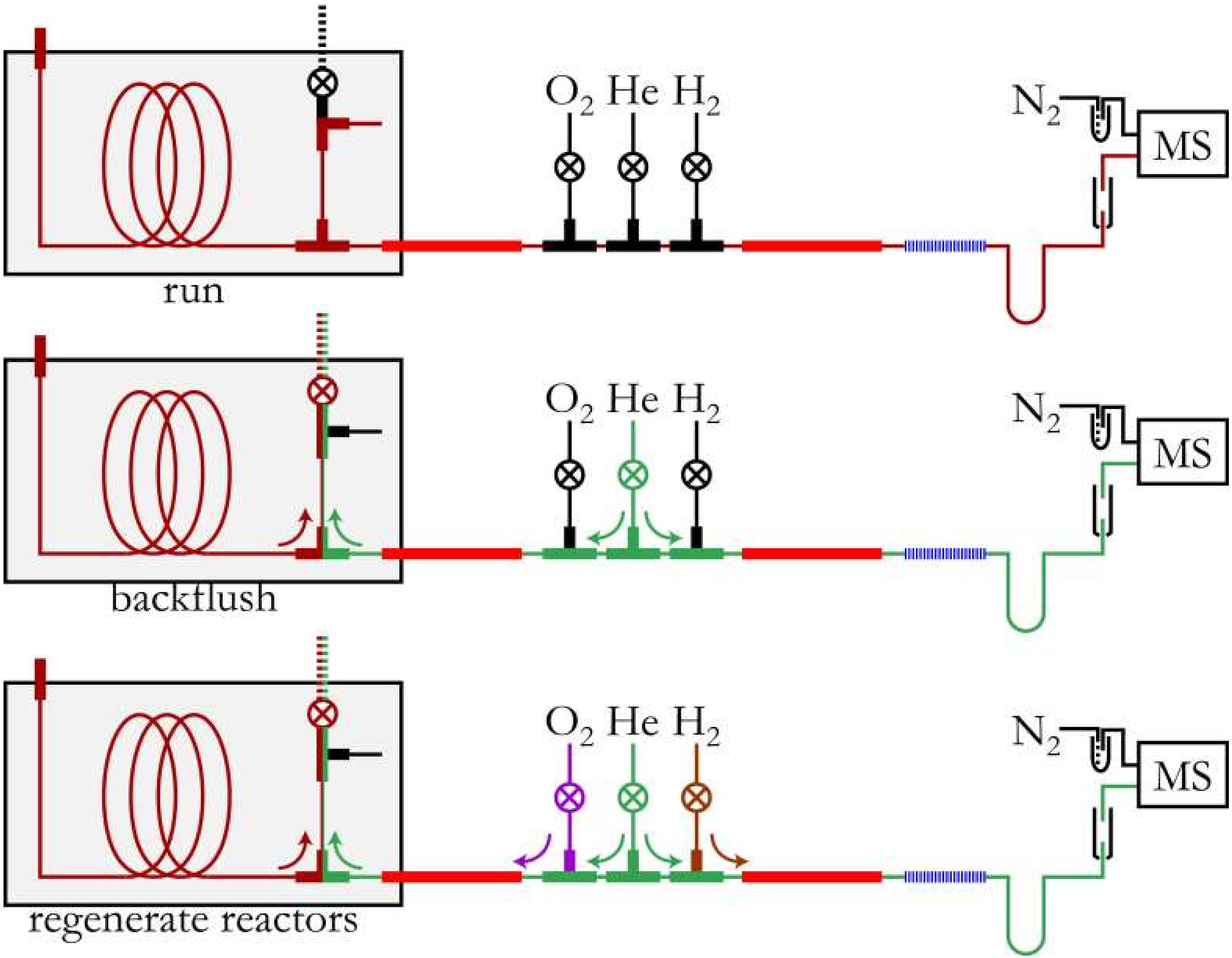
- CuO oxidizes Pt and Ni
- PtO and NiO produce O_2 for combustion
- $CNH_2 + O_2$ ---> $CO_2 + NO_x + H_2O$

Reduction at 650° C:

- $NO_x + Cu$ ---> $N_2 + CuO$

Nitrogen GC-IRMS




O2 He H2
N2
MS
run
O2 He H2
N2
MS
backflush
O2 He H2
N2
MS
regenerate reactors

Hydrogen GC-IRMS

- Reductive furnace
- Electrostatic lens for m/z 3 cup in MS (removes tail from ^{4}He)
- H_3^+ factor

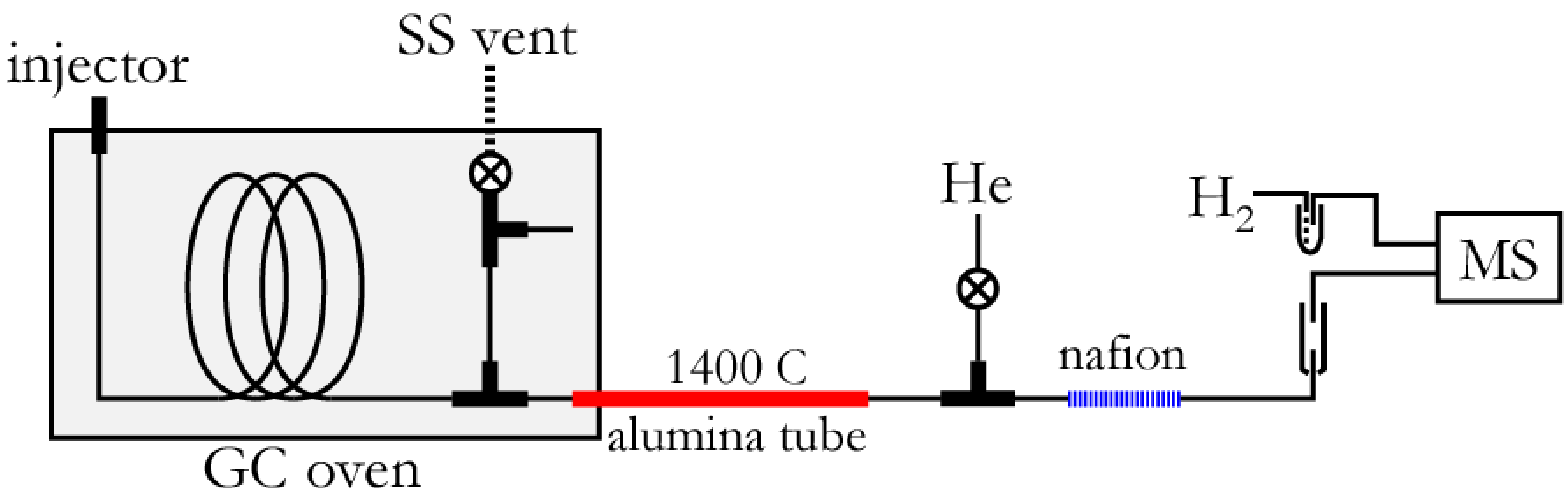
Conversion

$$H_xC_yN_zO_w$$

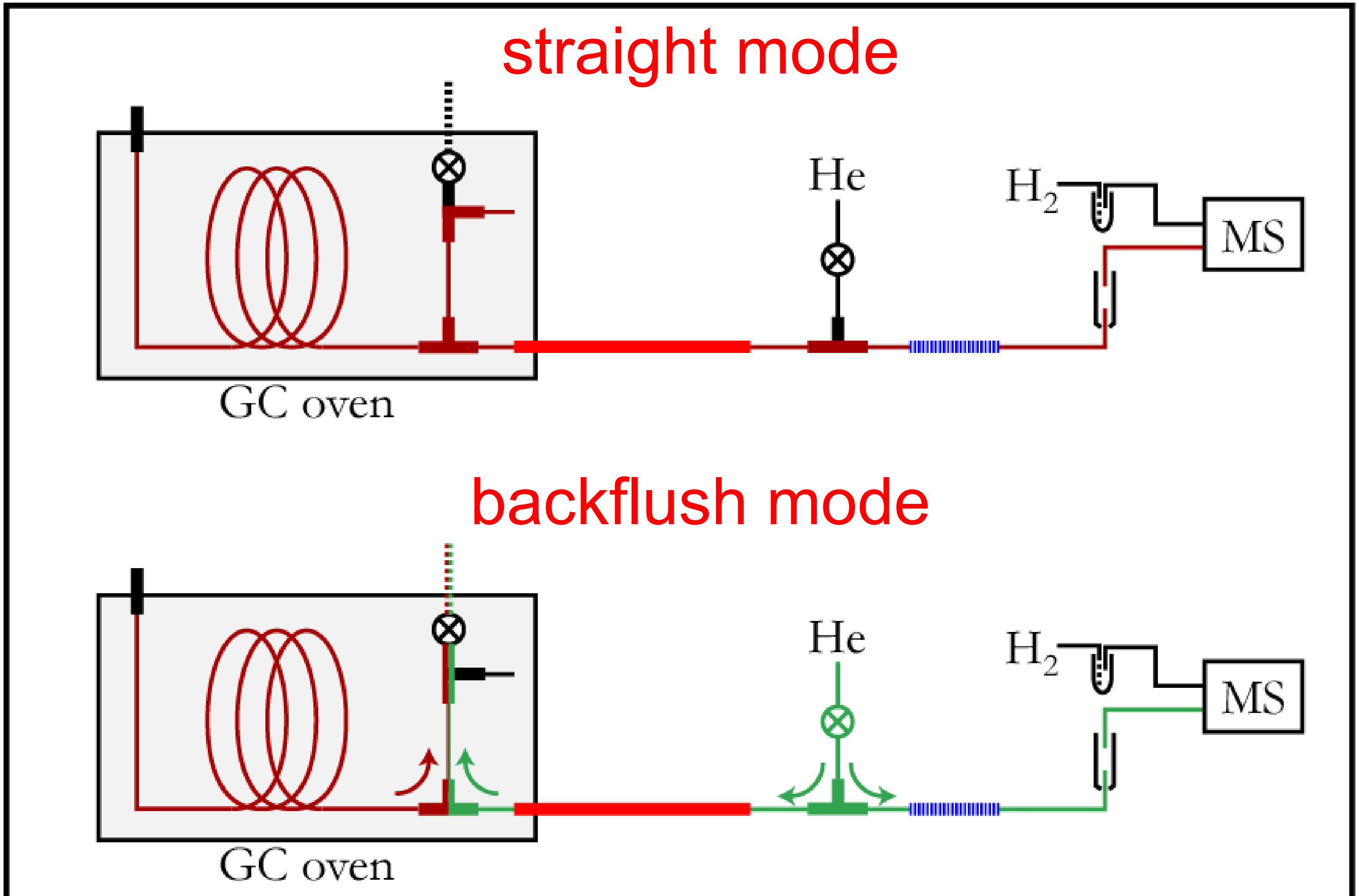
$$\xrightarrow{\Delta T}$$

$$x/2\ H_2 + w\ CO + (y\text{-}w)\ C + z/2\ N_2$$

Hydrogen GC-IRMS



Operation Modes

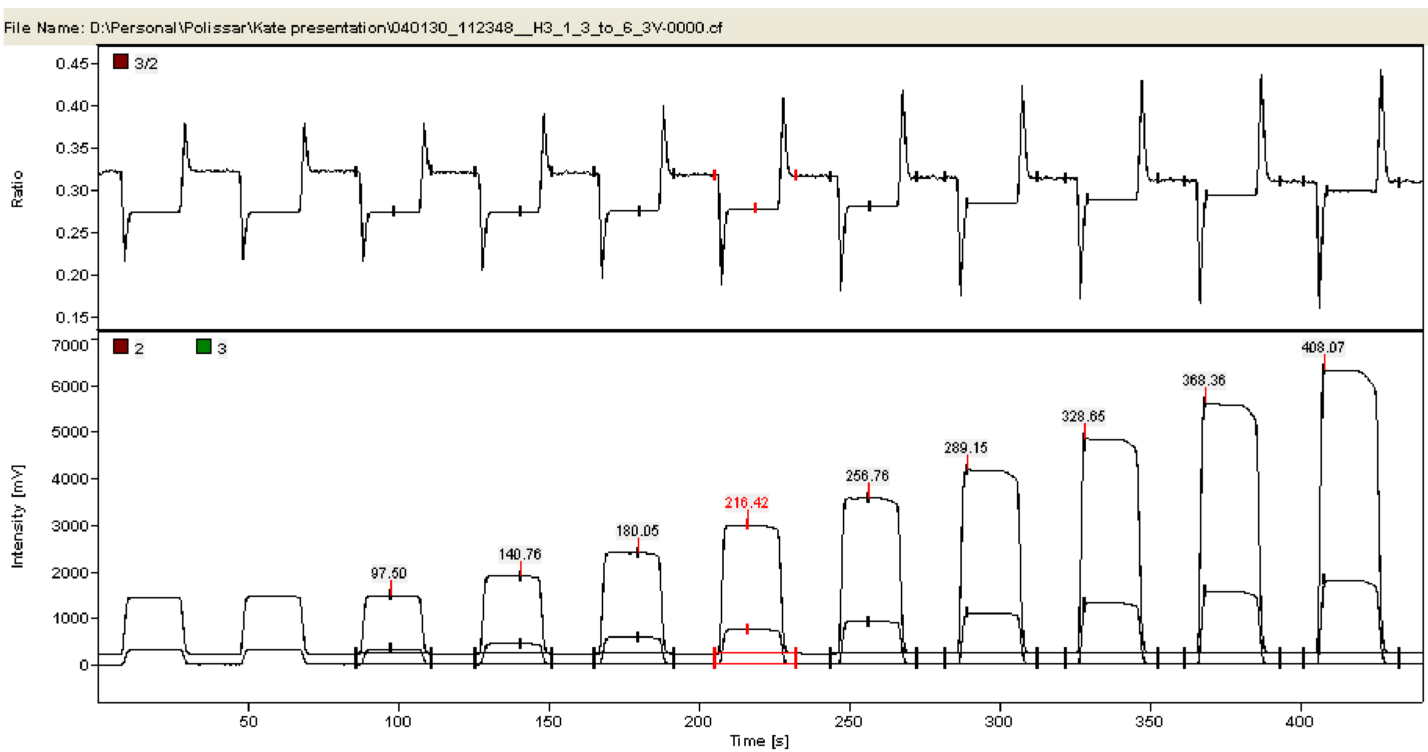


H_3^+ factor

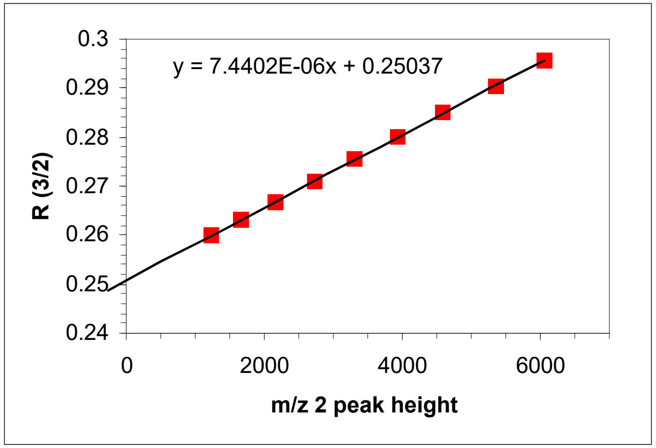
- In ion source H_3^+ is formed from H_2:
  $H_2^+ + H_2 \rightarrow H_3^+ + H$
- H_3^+ is isobaric with HD:
  m/z 3 = H_3^+ + HD
- Abundance of H_2^+ proportional to $[H_2]$
  Reaction is second order with $[H_2]$:
  $[H_3^+]=k\,[H_2]^2$

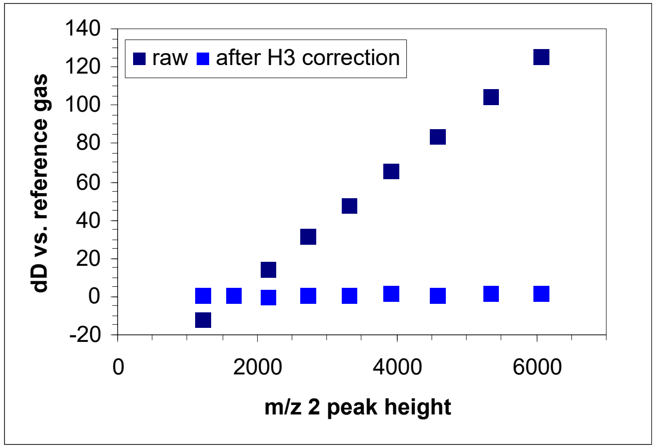
Determination of H_3^+ factor

- Run a series of reference gas pulses, varying gas pressure



Determination of H_3^+ factor

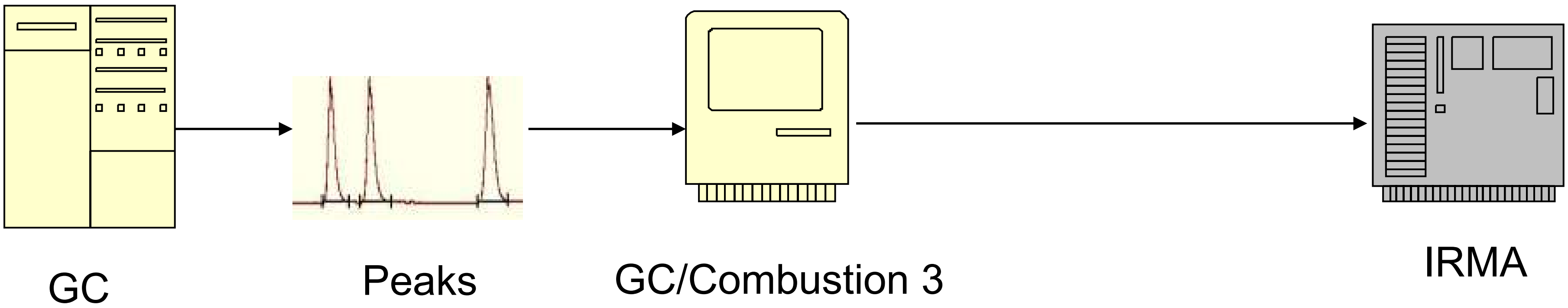




- Determine H_3^+ factor from relationship of peak height vs. R (m/z 3/2 ratio)
- Slope = H_3^+ factor
- Intercept = 3/2 ratio of gas
- Relatively large correction (10's per-mil)

Isotope ratio analysis

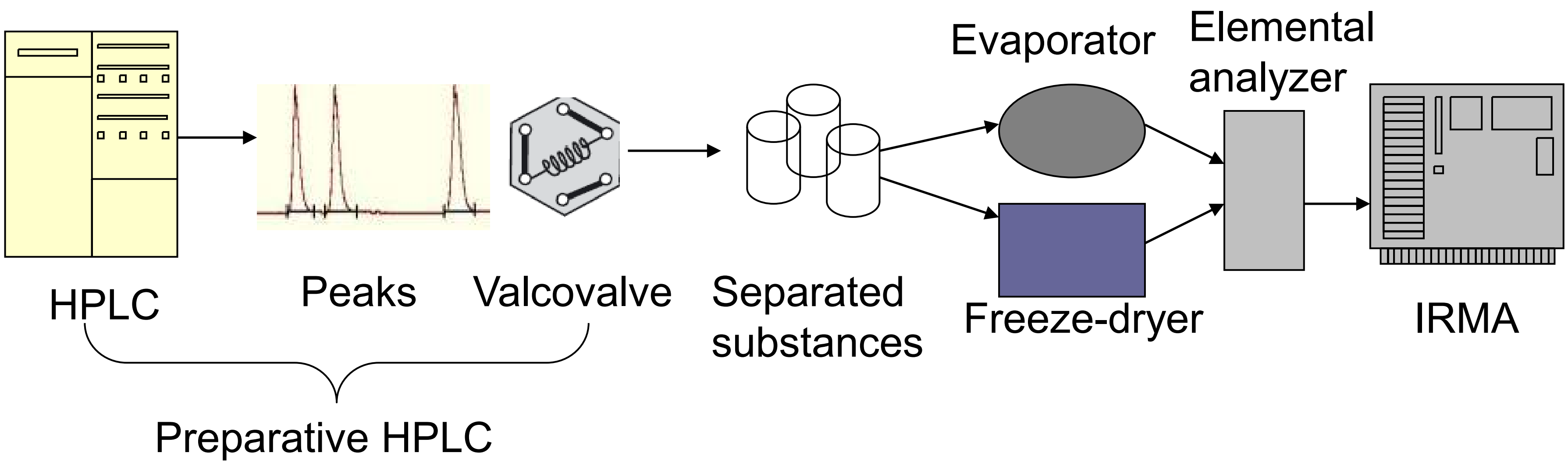
- GC-Combustion for volatile compounds



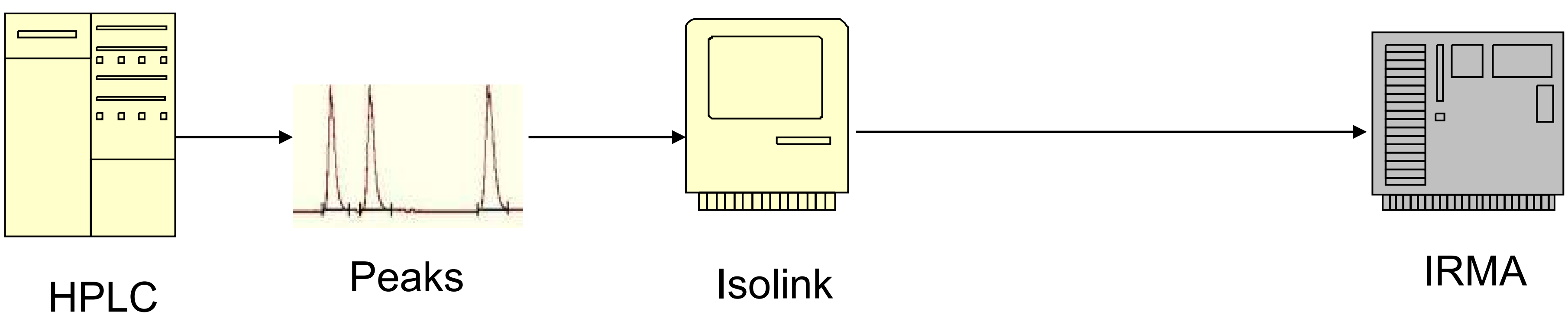
However, not for compounds with
High molecular weigth
High polarity
Thermal instability

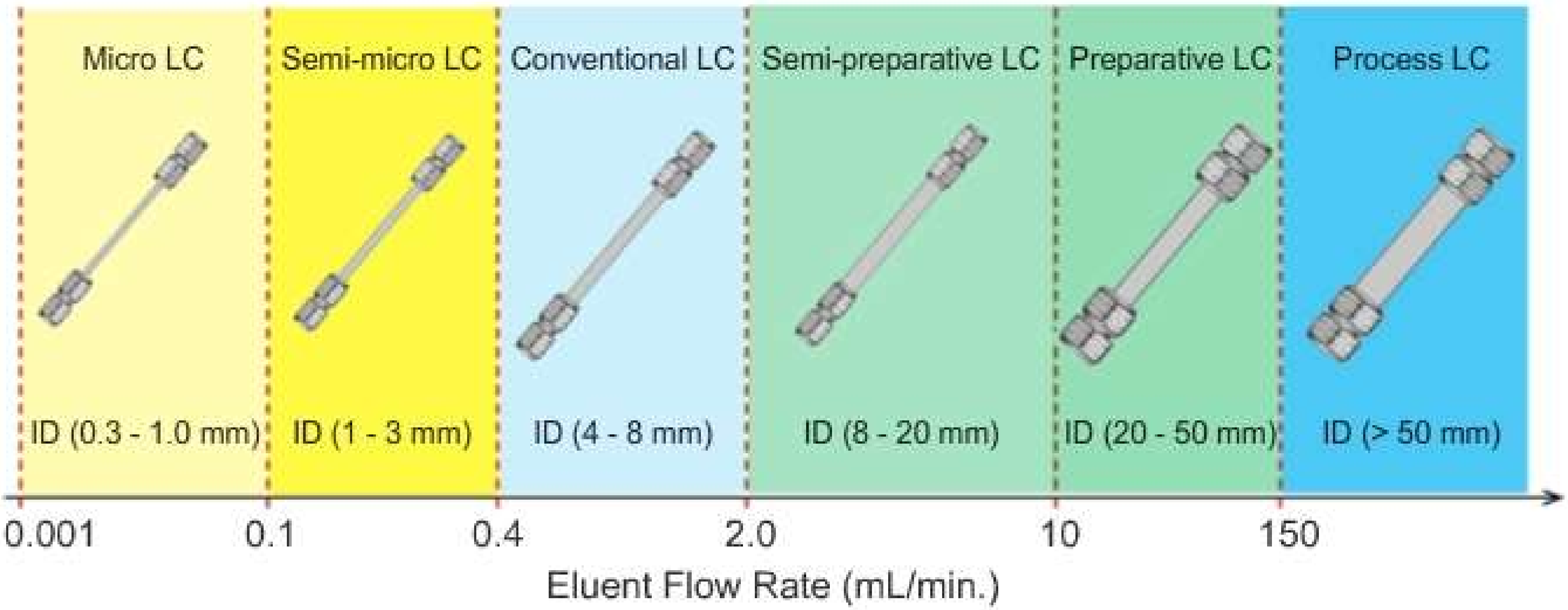
Isotope ratio analysis

Classic System (LC+irMS)

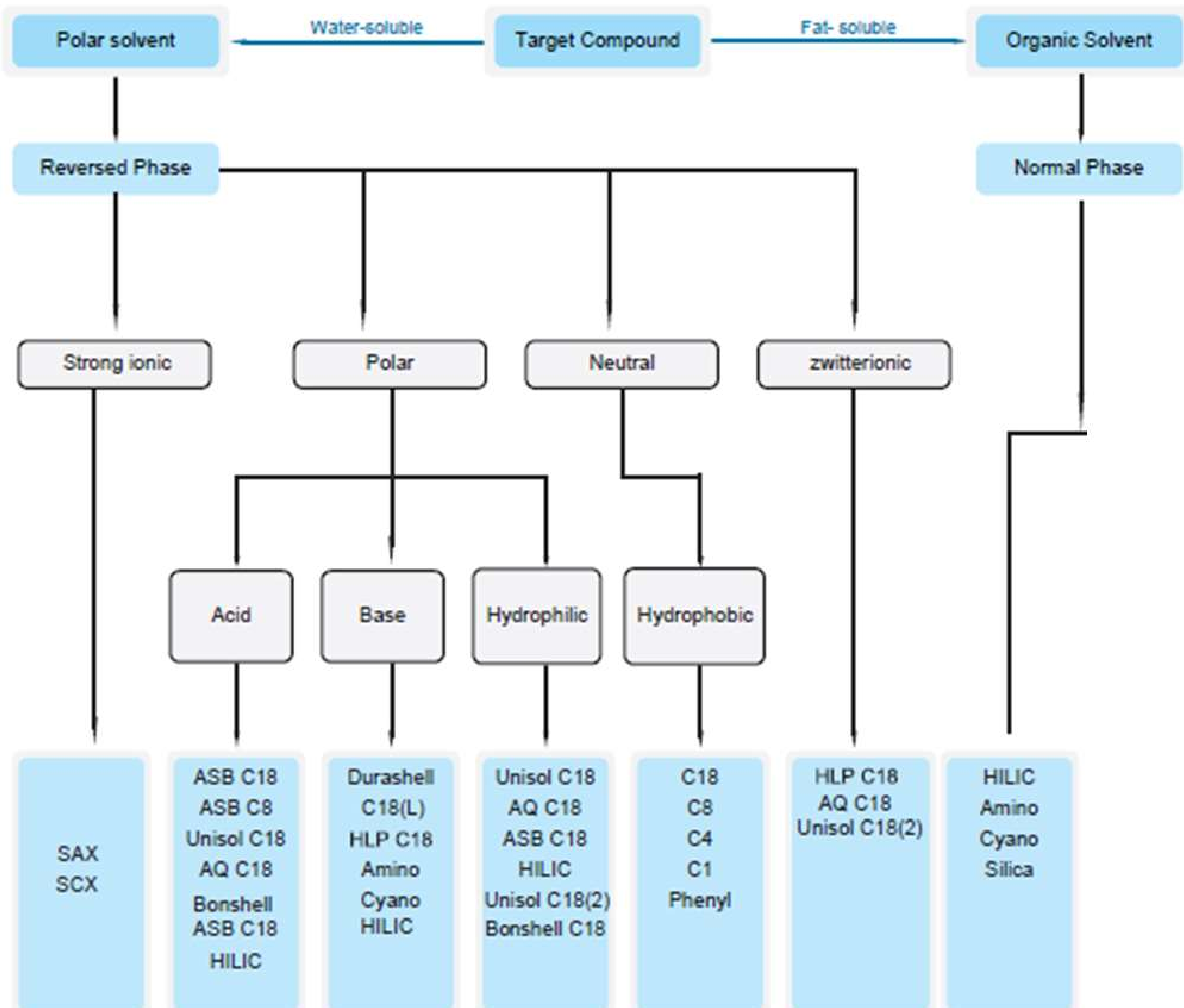


New System (LC-IRMS)

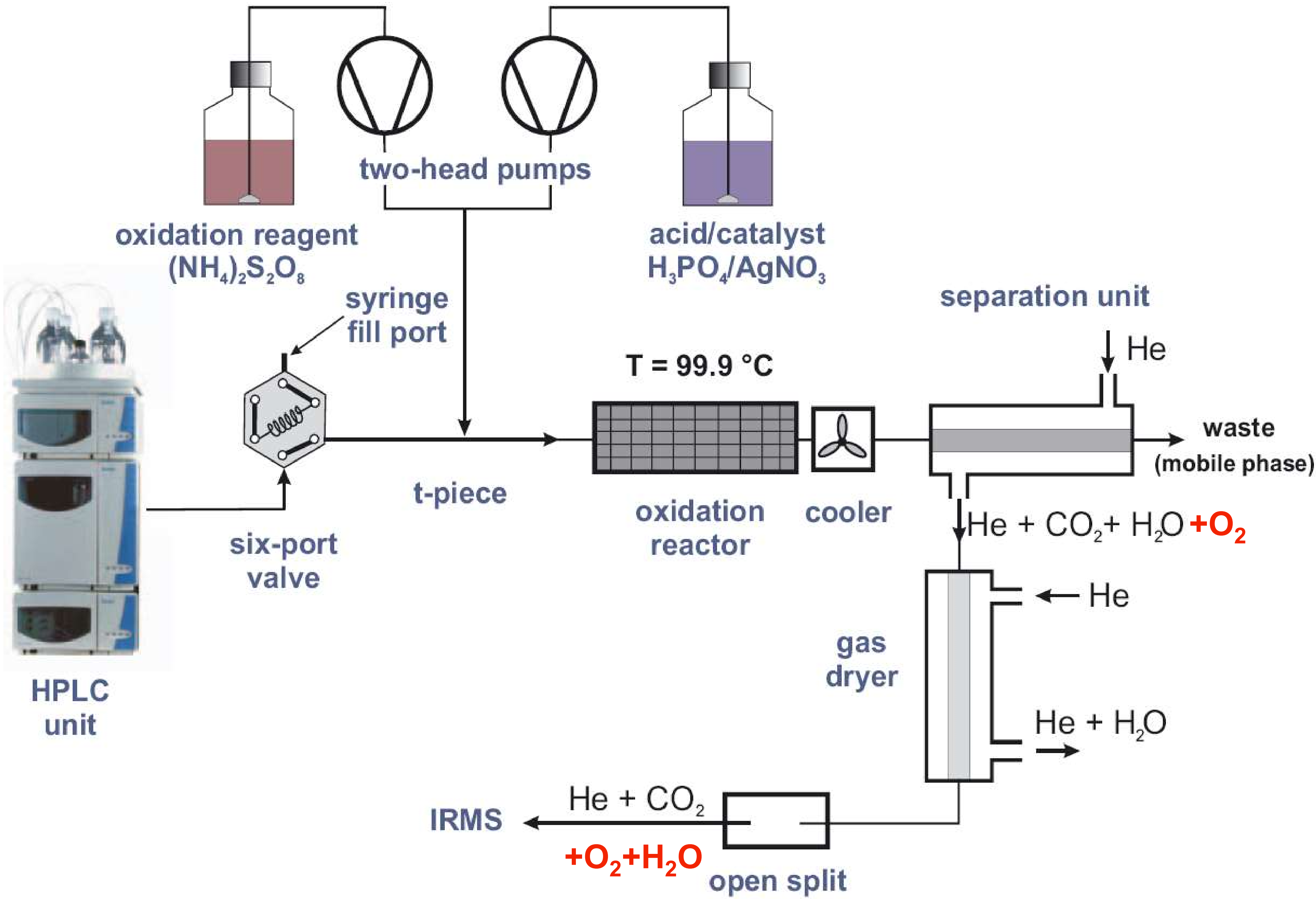




Micro LC
Semi-micro LC
Conventional LC
Semi-preparative LC
Preparative LC
Process LC
ID (0.3 - 1.0 mm)
ID (1 - 3 mm)
ID (4 - 8 mm)
ID (8 - 20 mm)
ID (20 - 50 mm)
ID (> 50 mm)
0.001
0.1
0.4
2.0
10
150
Eluent Flow Rate (mL/min.)

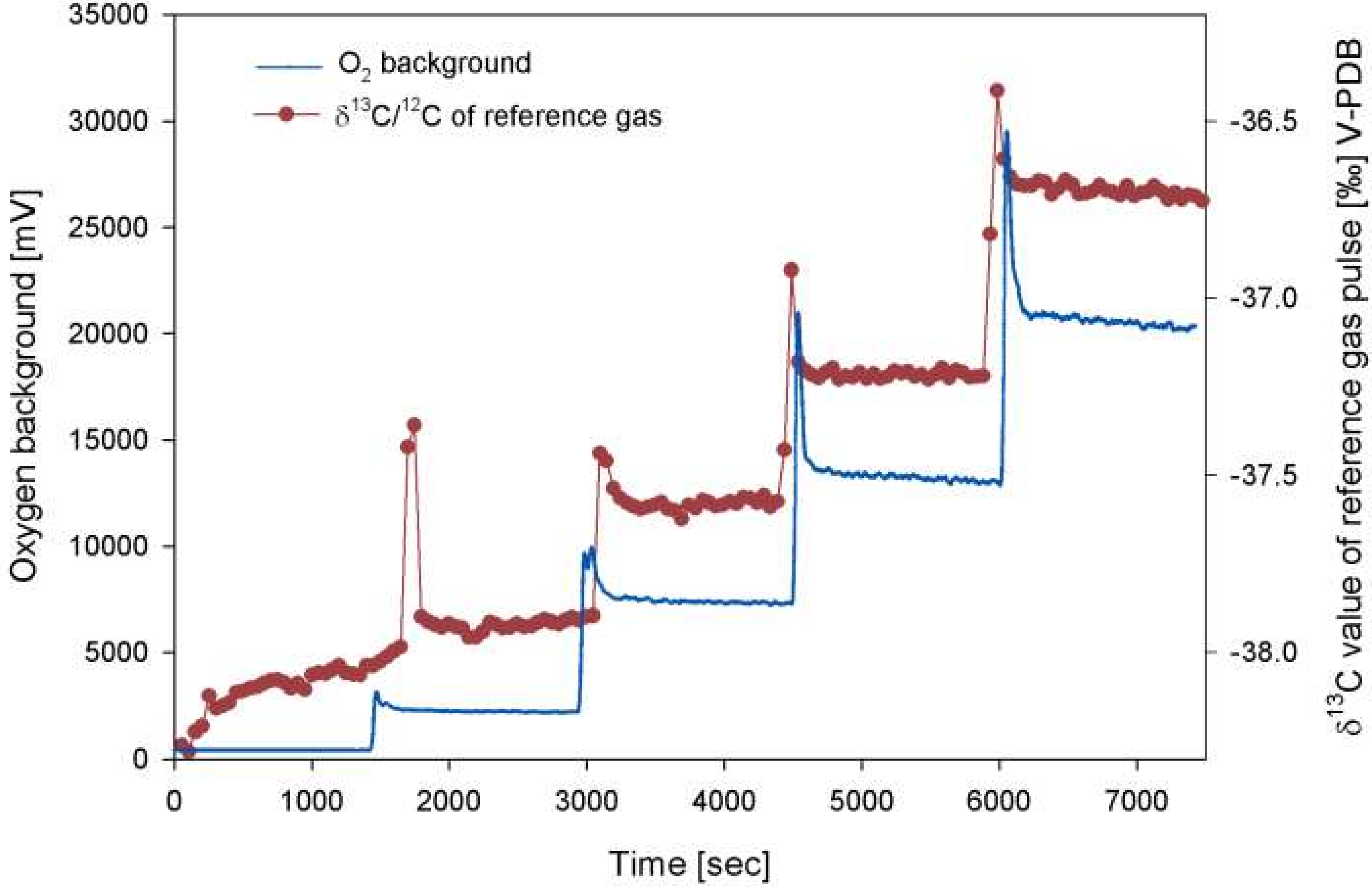


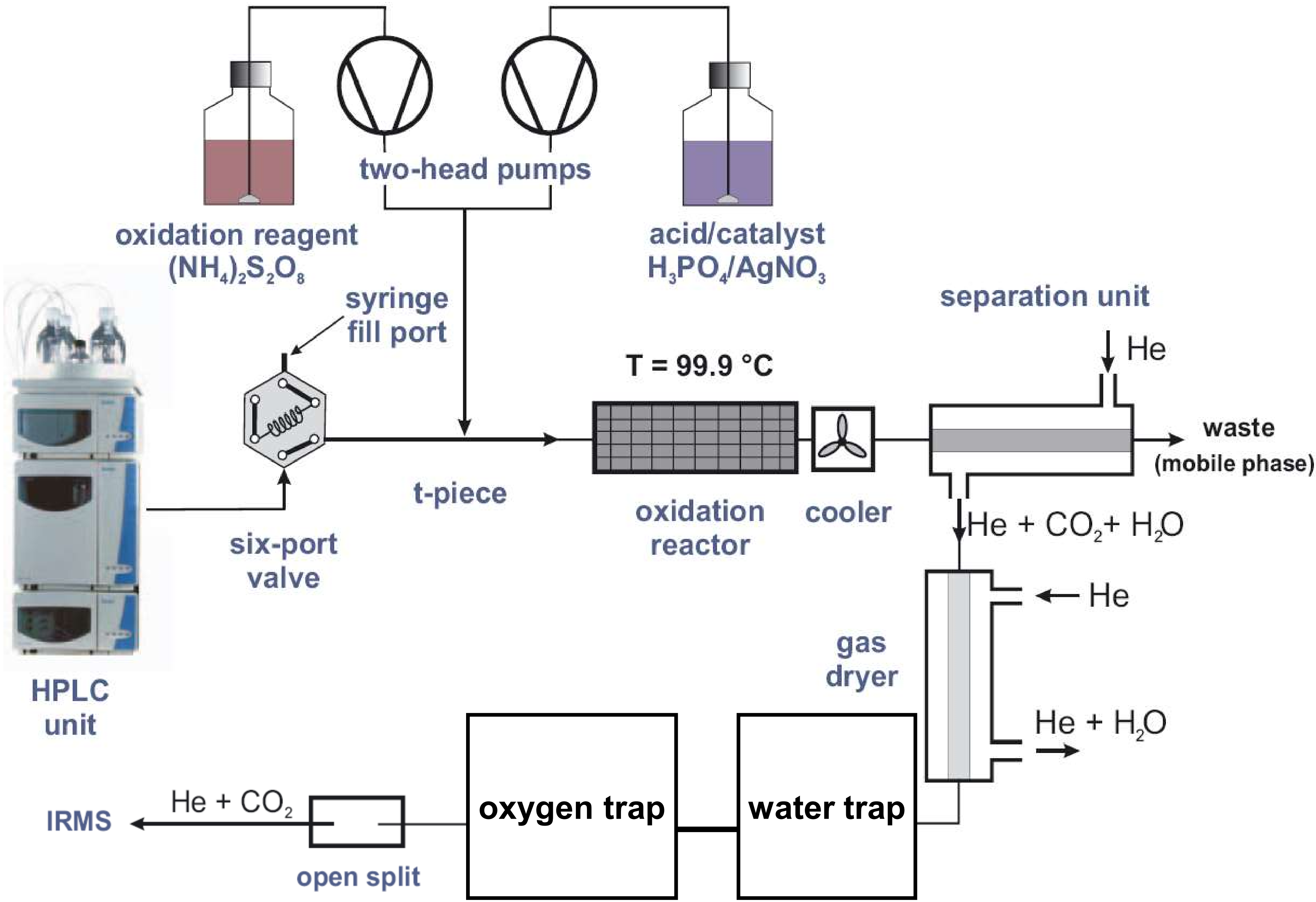
Polar solvent
Water-soluble
Target Compound
Fat- soluble
Organic Solvent
Reversed Phase
Normal Phase
Strong ionic
Polar
Neutral
zwitterionic
Acid
Base
Hydrophilic
Hydrophobic
SAX
SCX
ASB C18
ASB C8
Unisol C18
AQ C18
Bonshell ASB C18
HILIC
Durashell C18(L)
HLP C18
Amino
Cyano
HILIC
Unisol C18
AQ C18
ASB C18
HILIC
Unisol C18(2)
Bonshell C18
C18
C8
C4
C1
Phenyl
HLP C18
AQ C18
Unisol C18(2)
HILIC
Amino
Cyano
Silica



two-head pumps
oxidation reagent
(NH4)2S2O8
acid/catalyst
H3PO4/AgNO3
syringe
fill port
separation unit
He
T = 99.9 °C
waste
(mobile phase)
t-piece
oxidation
reactor
cooler
six-port
valve
He + CO2+ H2O +O2
He
gas
dryer
HPLC
unit
He + H2O
He + CO2
IRMS
+O2+H2O
open split

O_2 background to measured $\delta^{13}C$ values of reference gas pulse

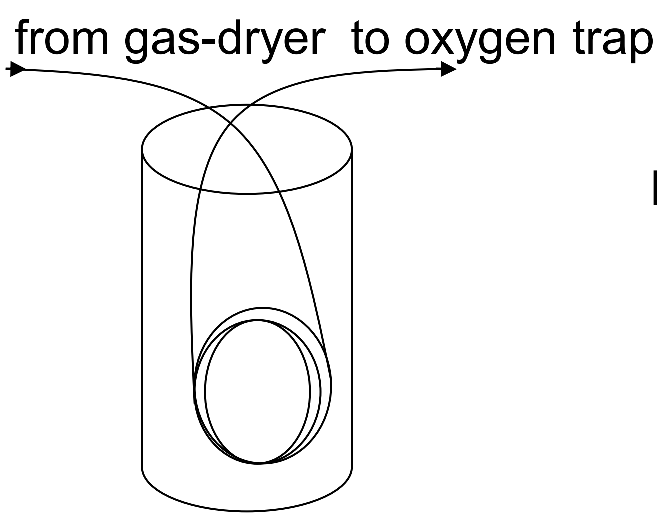


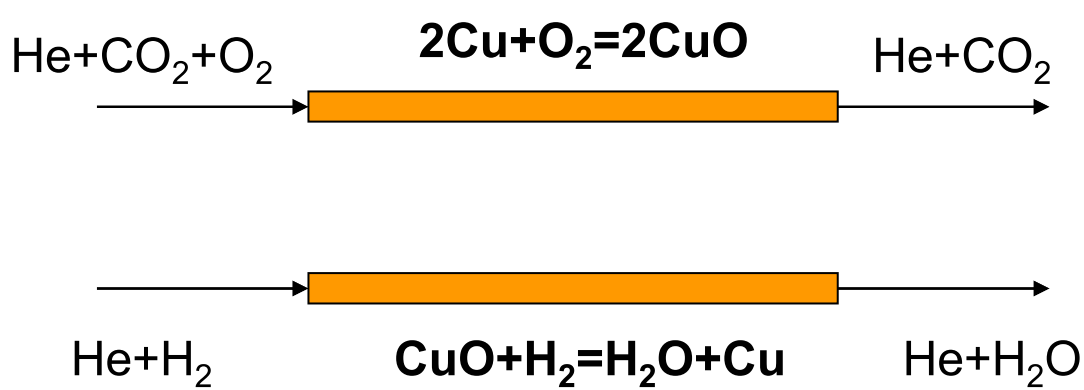


two-head pumps
oxidation reagent
$(NH_4)_2S_2O_8$
acid/catalyst
$H_3PO_4/AgNO_3$
syringe
fill port
separation unit
He
T = 99.9 °C
waste
(mobile phase)
t-piece
six-port
valve
oxidation
reactor
cooler
He + CO_2 + H_2O
He
gas
dryer
HPLC
unit
He + H_2O
He + CO_2
IRMS
oxygen trap
water trap
open split

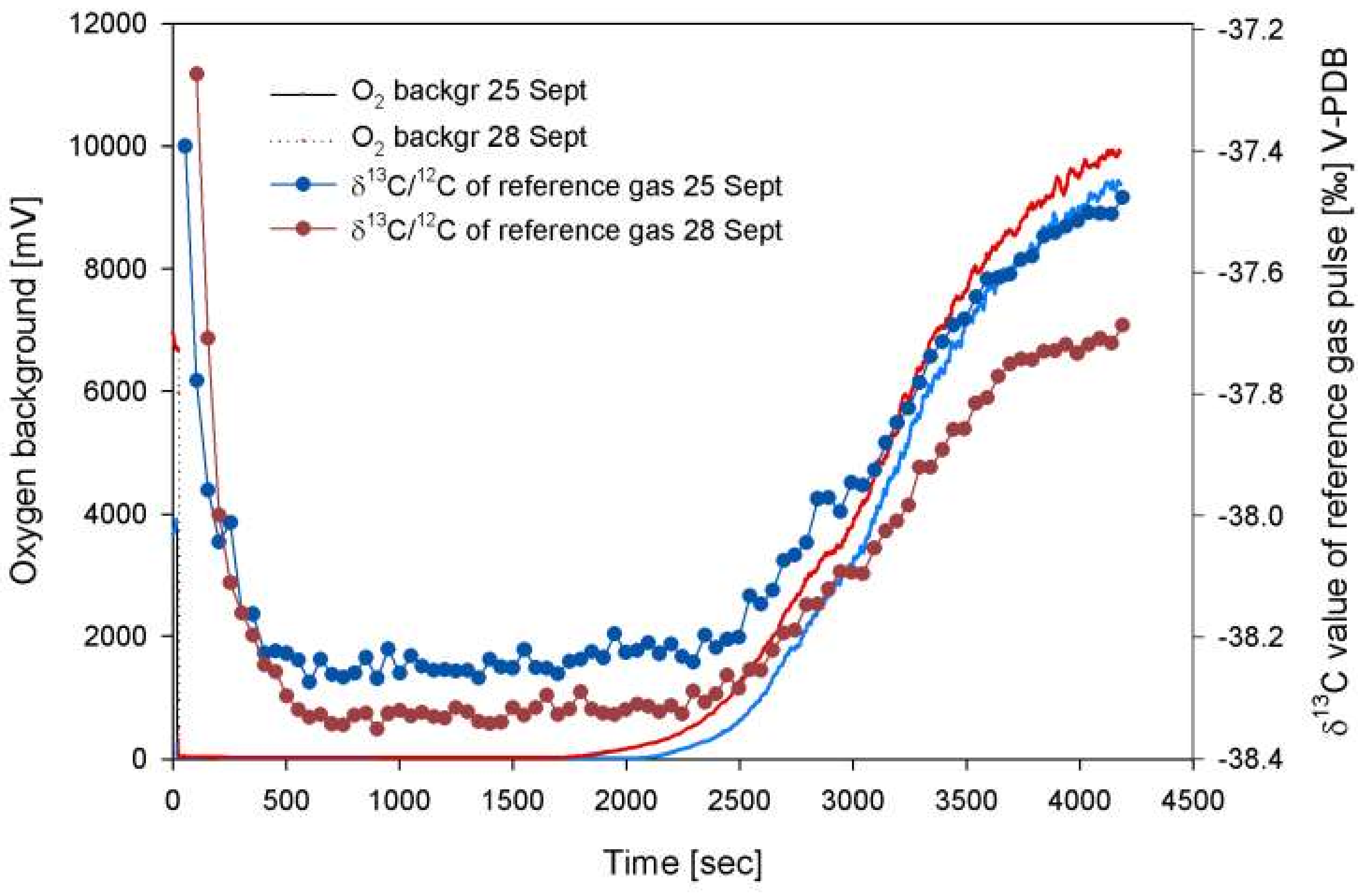
Traps

- Water trap
  - Long capillare
  - Dry ice
- Oxygen trap
  - 580° C
  - 2 ceramic tubes filled with Cu wire
  - 8 port valve
  - $He:H_2$ (98:2)

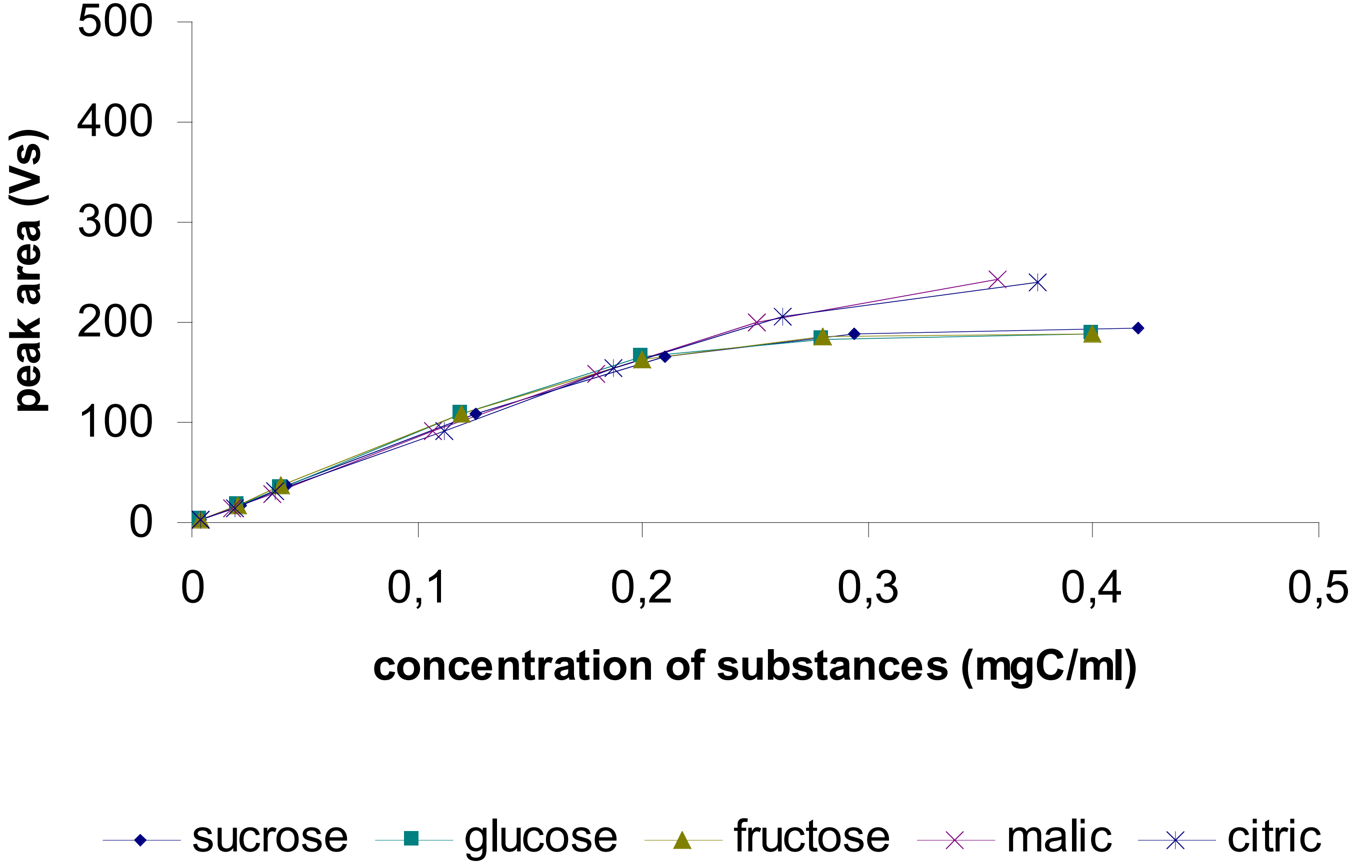




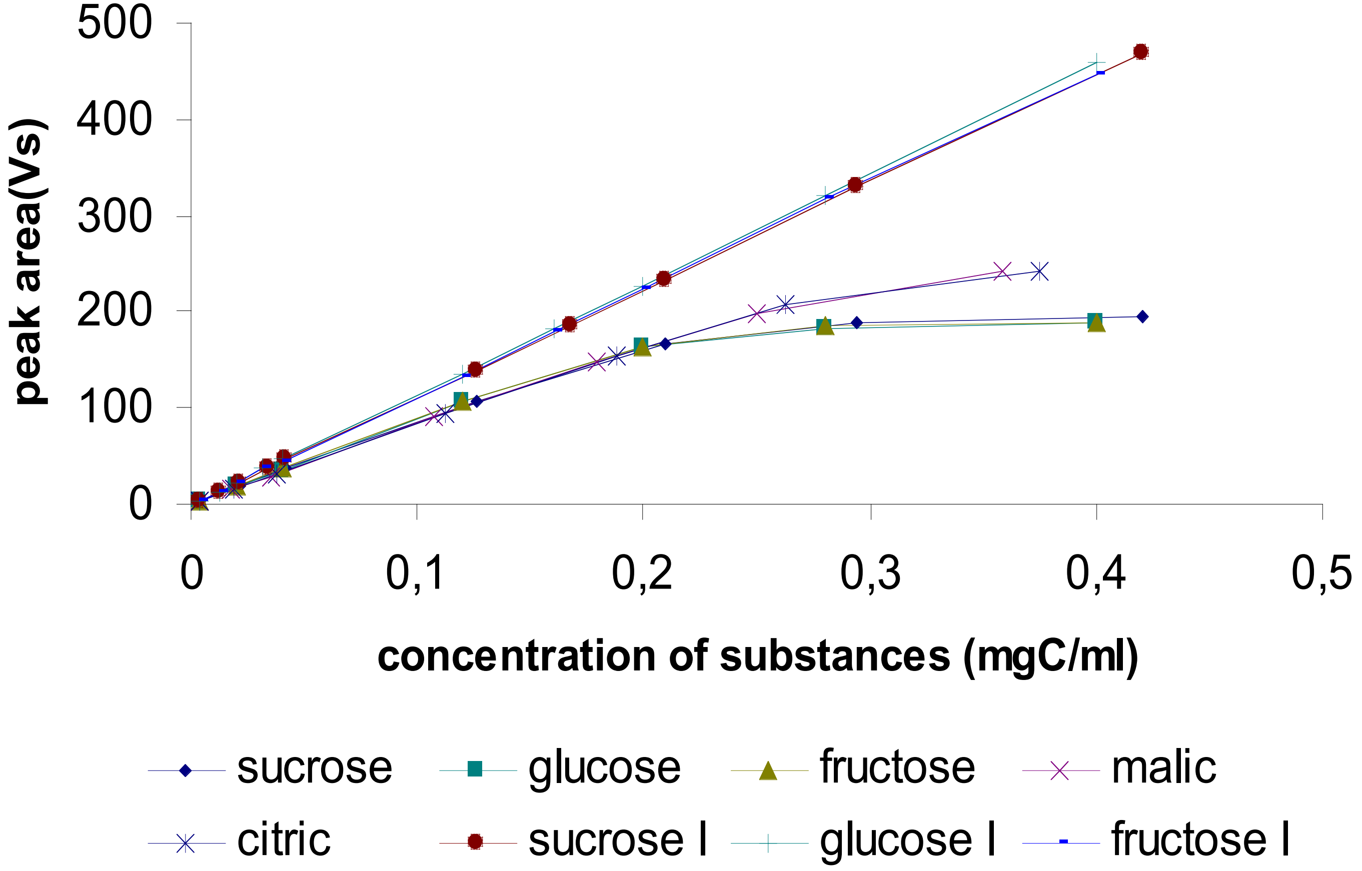
O_2 background



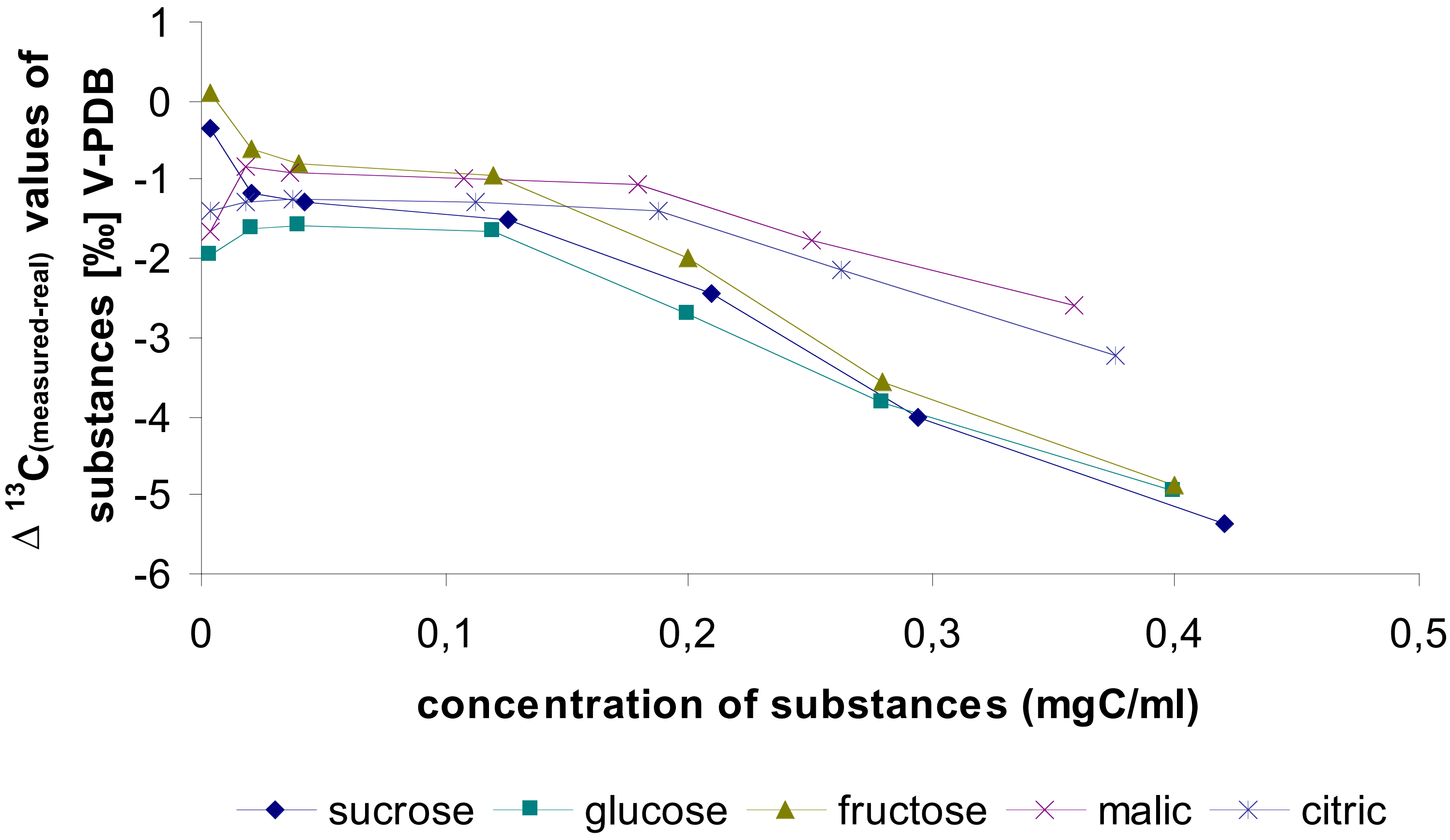
Linearity and reproducibility of the system



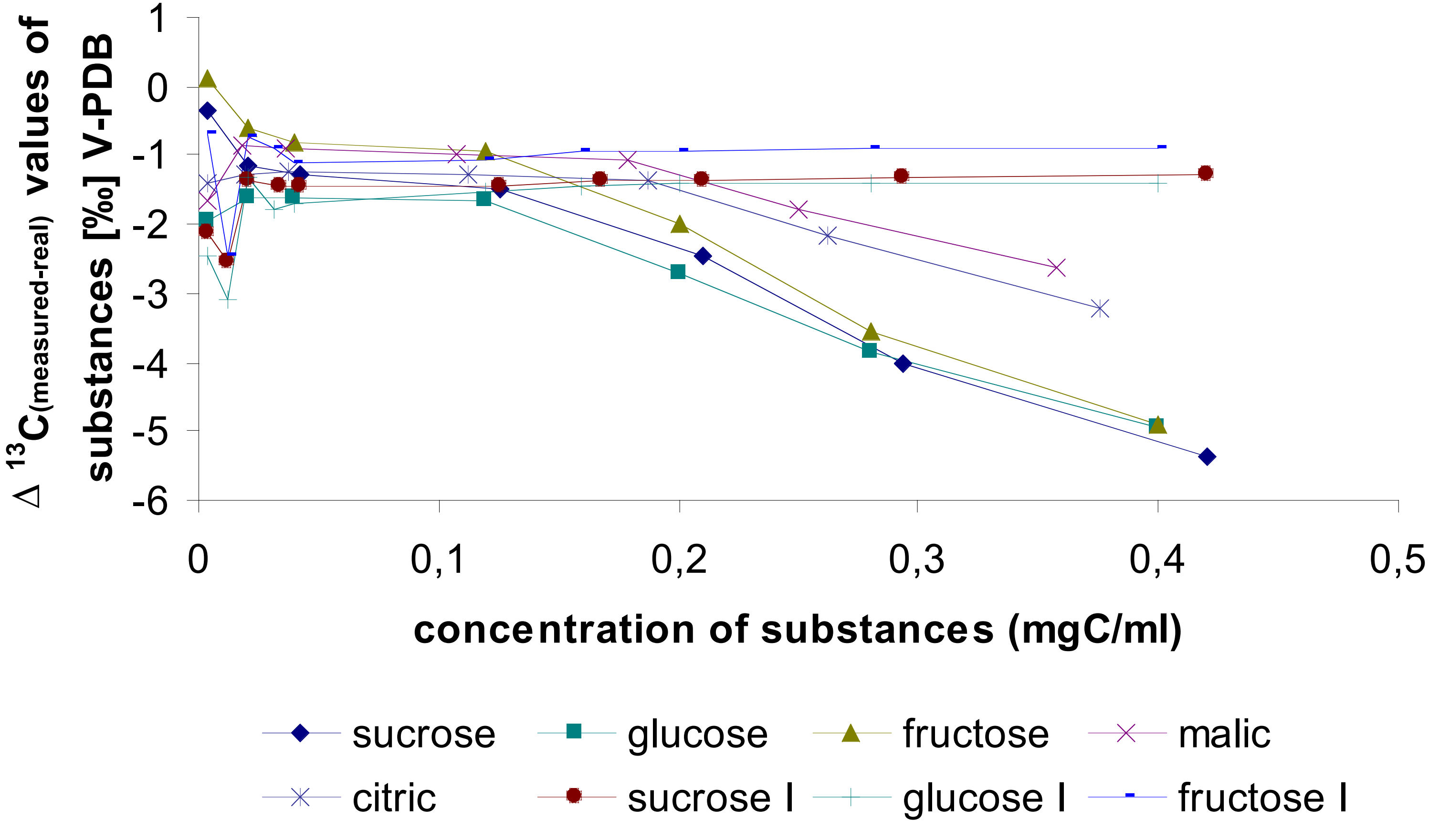
Linearity and reproducibility of the system



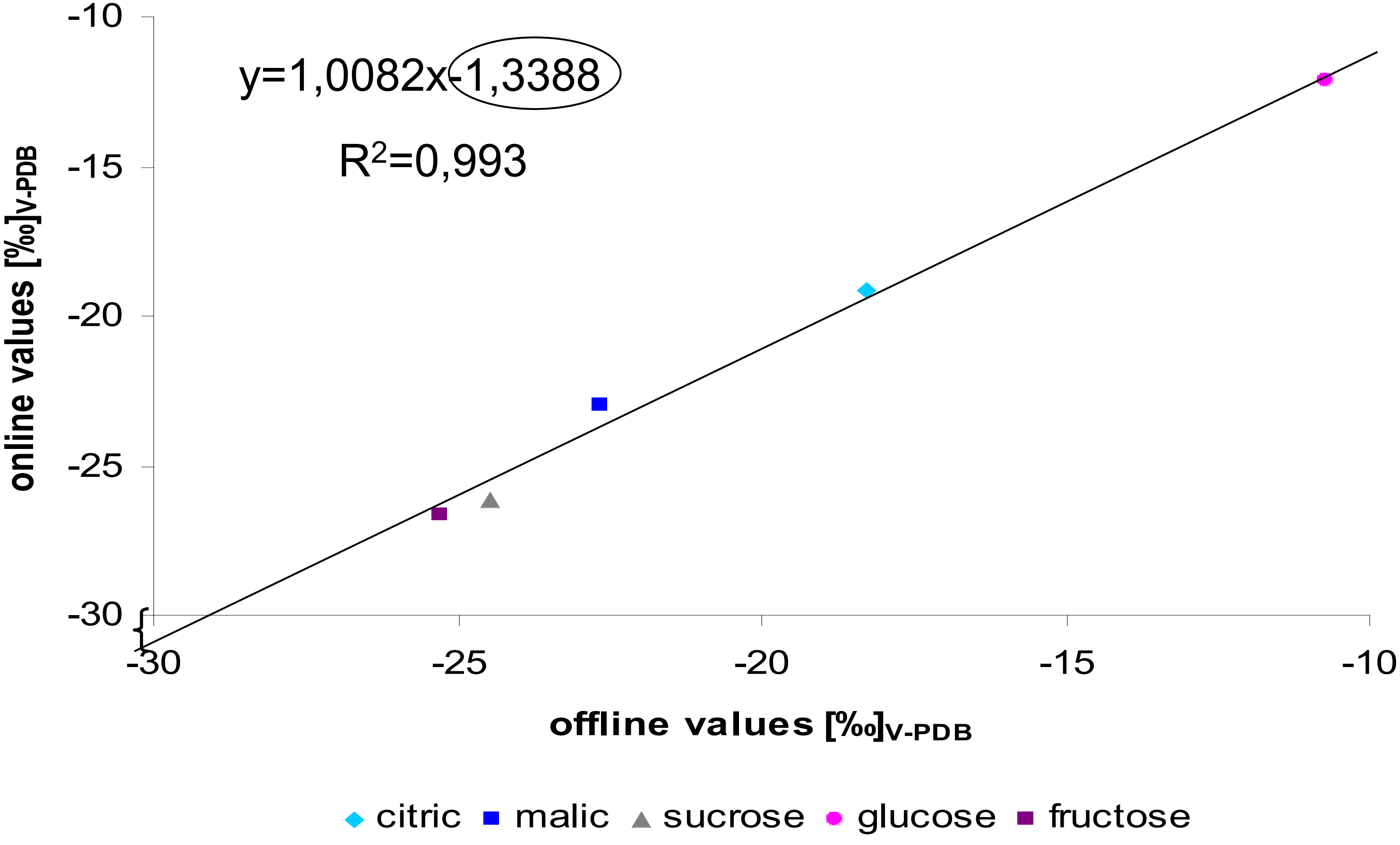
Linearity and reproducibility of the system



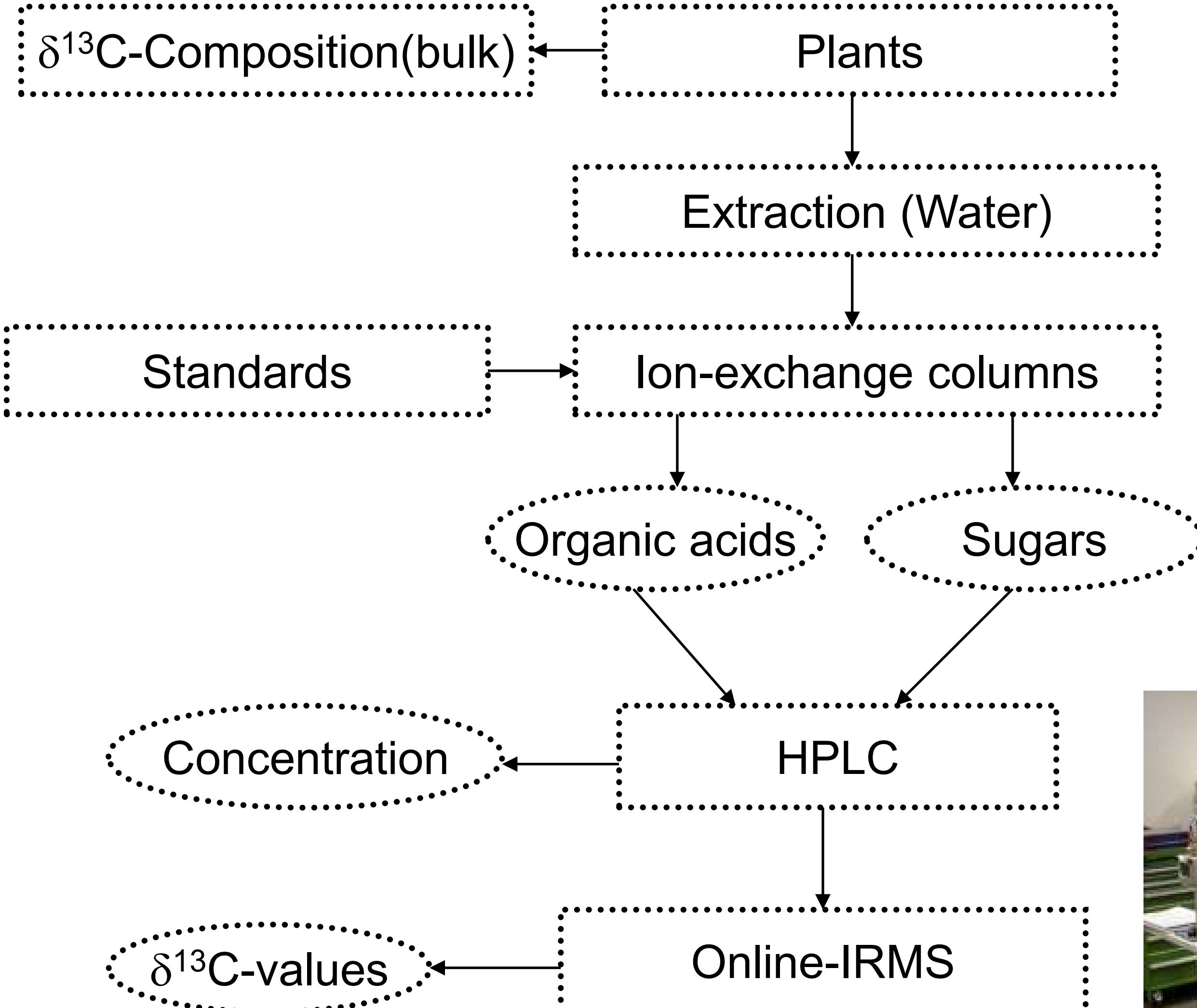
Linearity and reproducibility of the system



Accuracy of the system



Isolation of plant metabolites

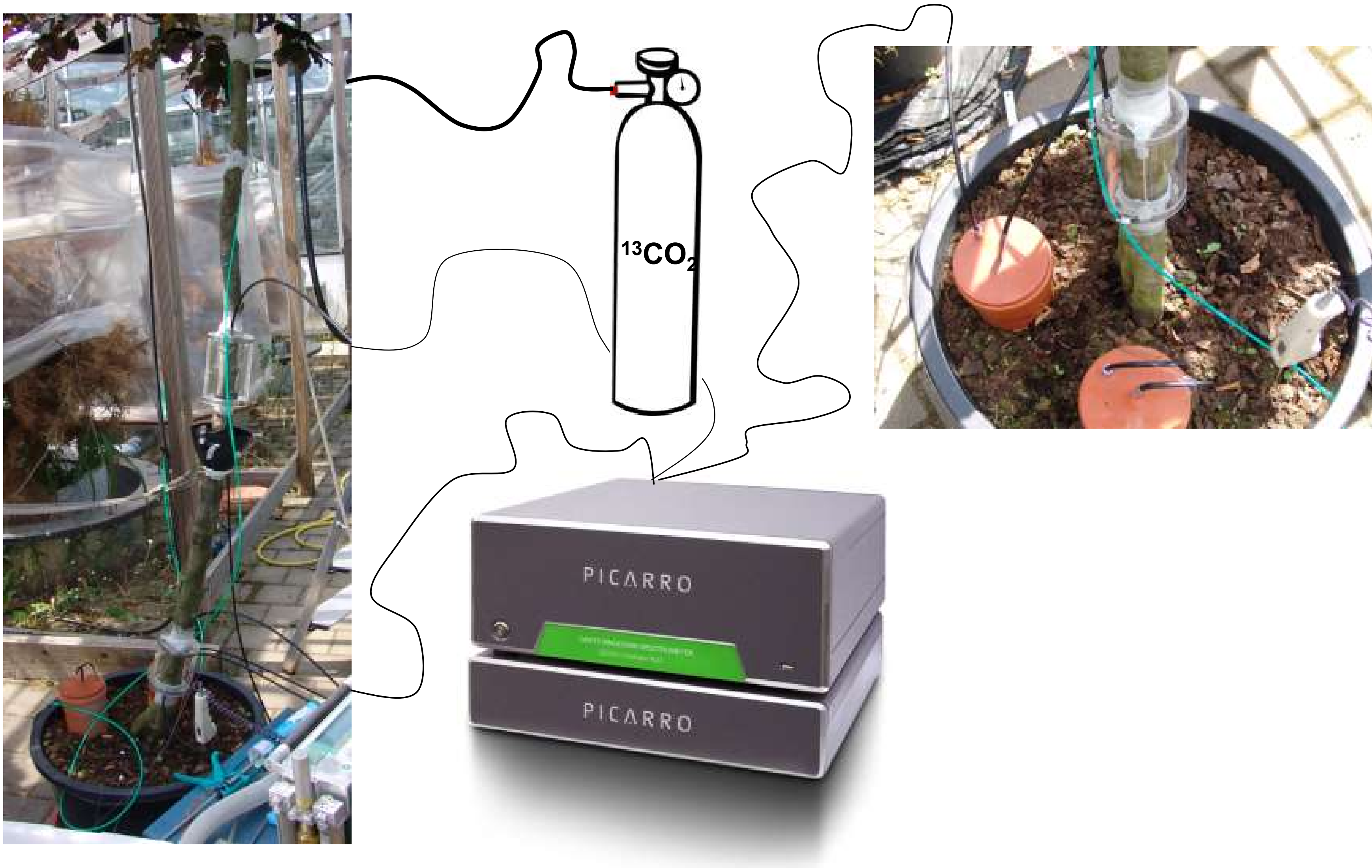


Molecular Biogeochemistry

Gerd Gleixner

Max-Planck-Institut
für Biogeochemie, Jena

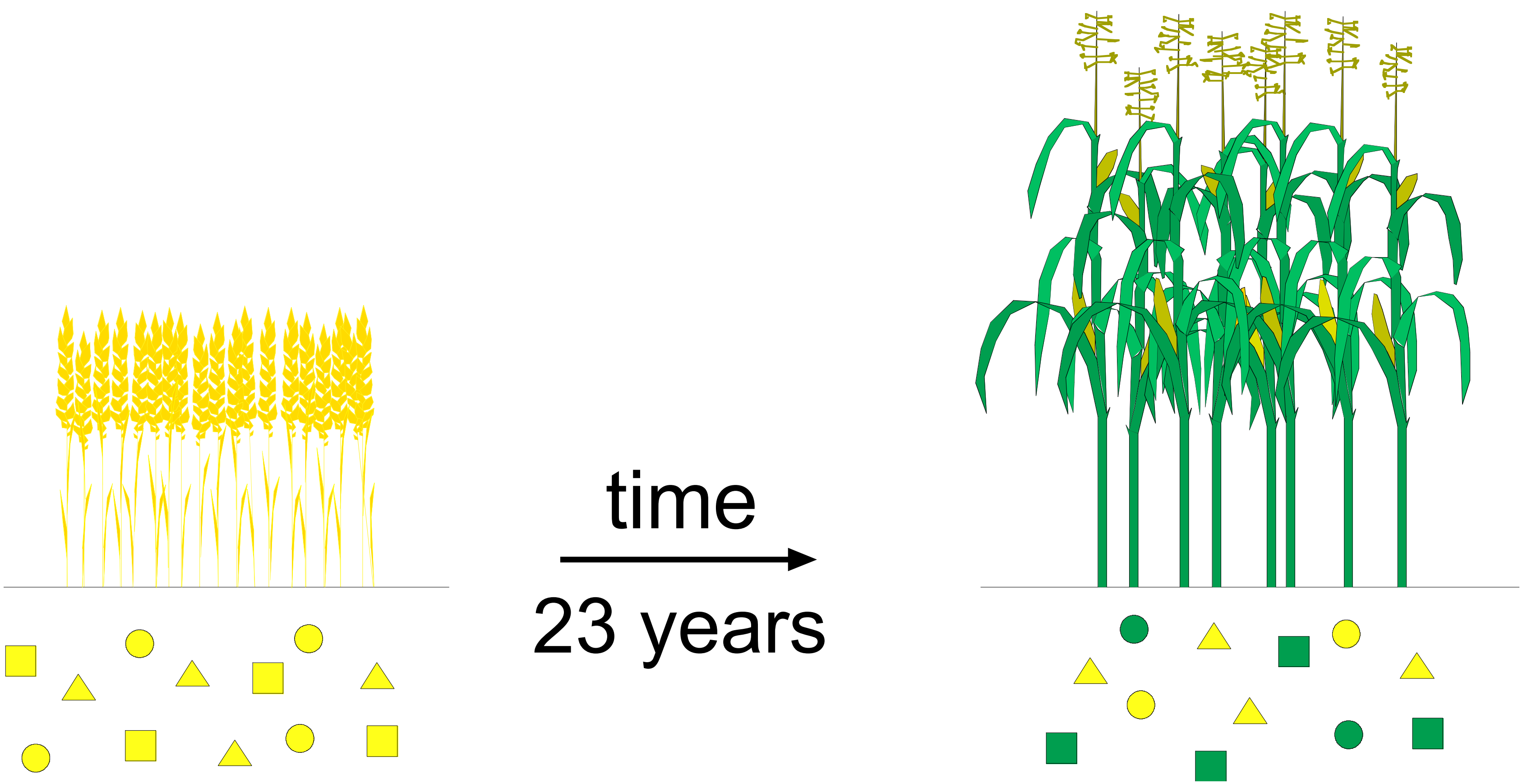

PICARRO

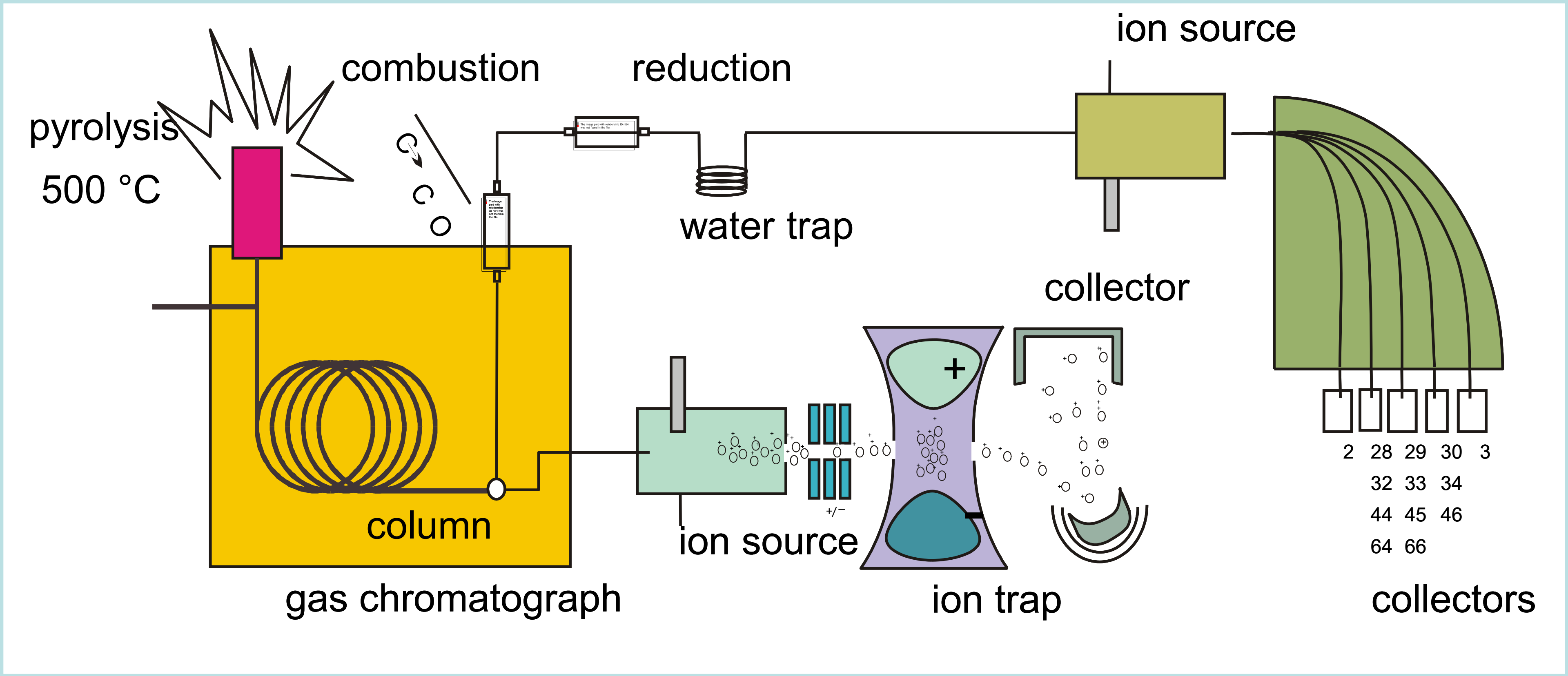

$^{13}CO_2$
PICARRO
PICARRO

Natural Labeling Experiment

$\delta^{13}C$ □ -25‰

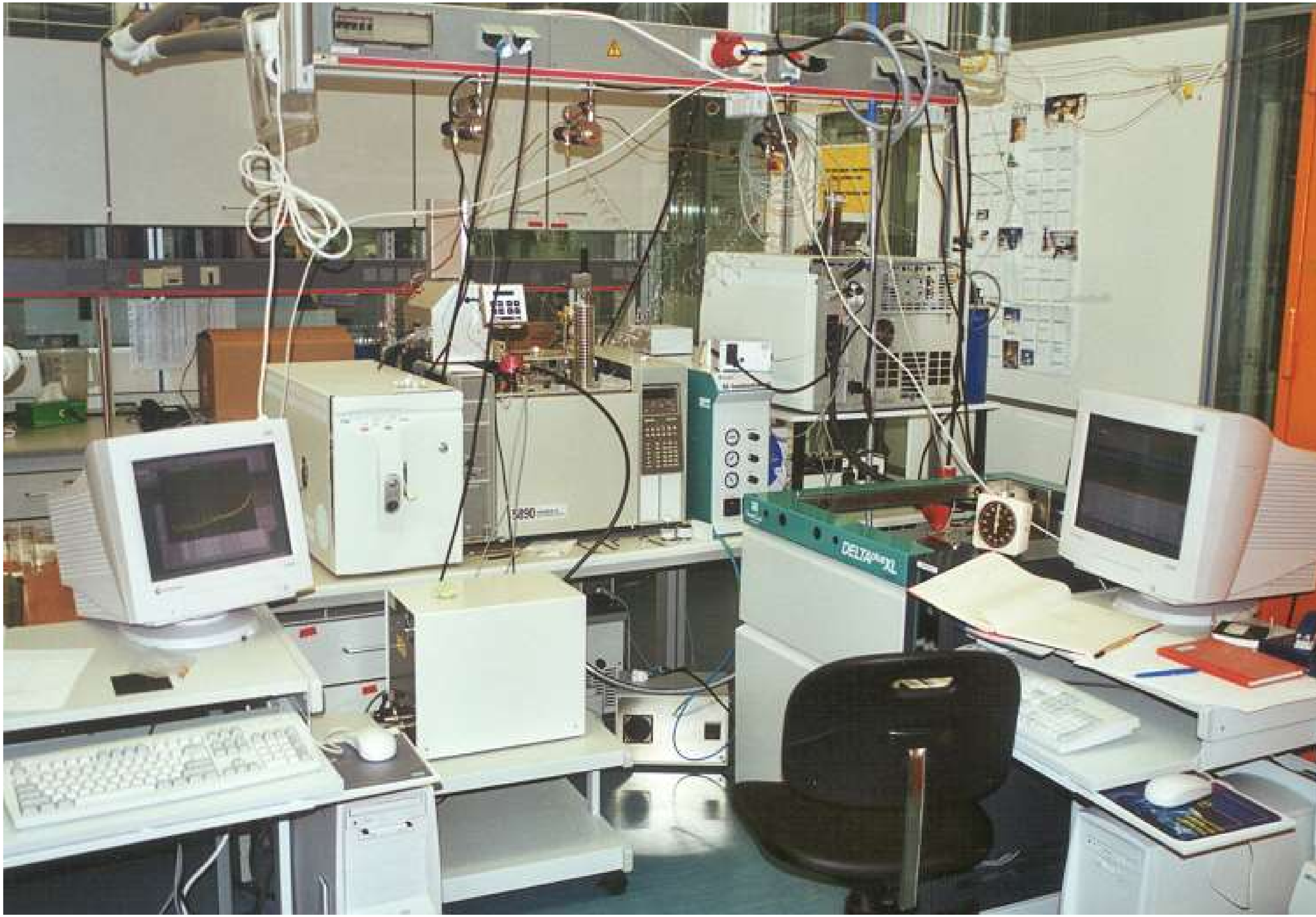
$\delta^{13}C$ □ -12‰



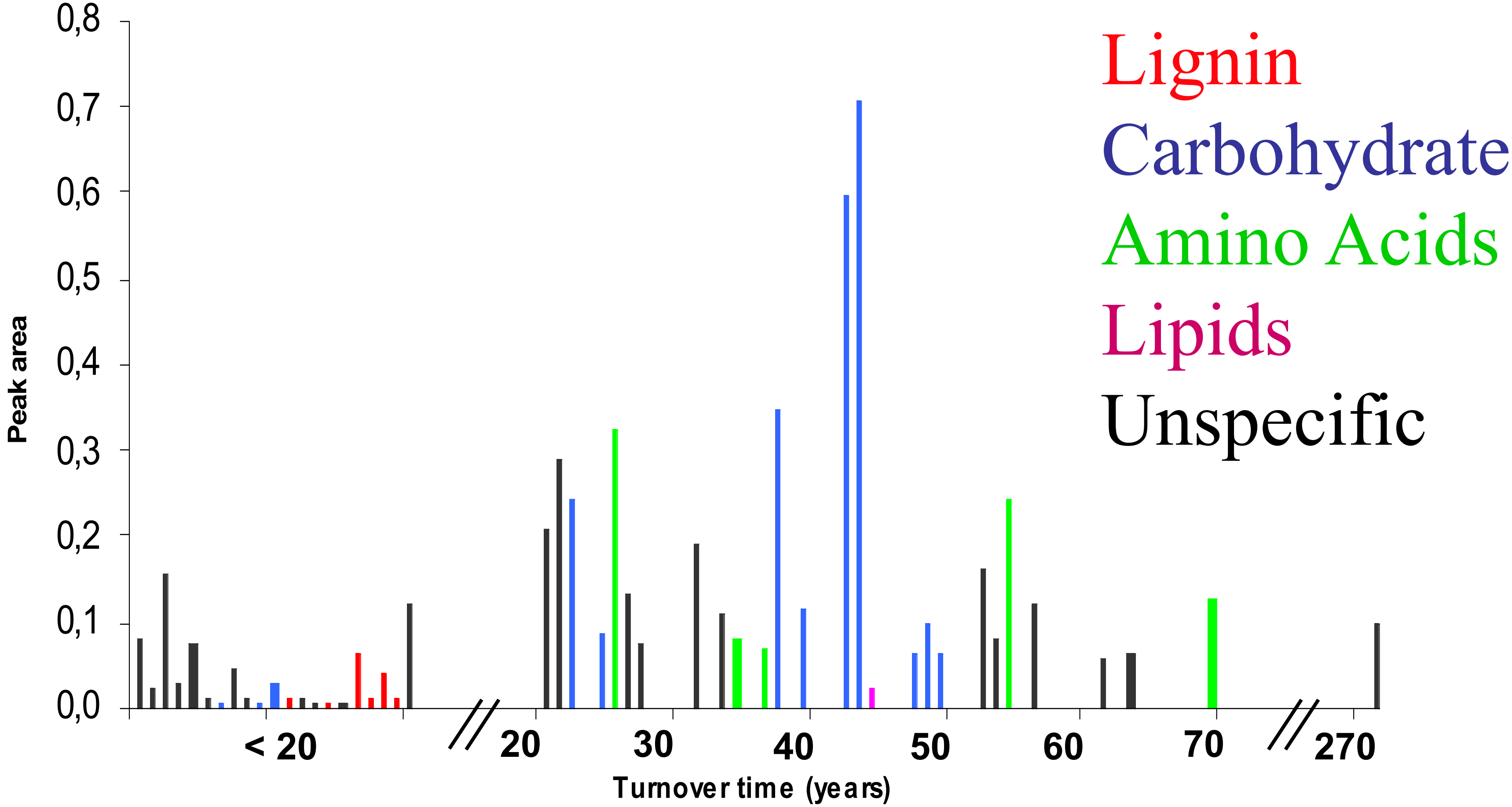


Precursor	Pyrolysis Products
Carbohydrate	Acetic Acid, Furanes
Protein	Nitriles, Pyrroles
Lignin	Phenols

after Gleixner et al., 1999

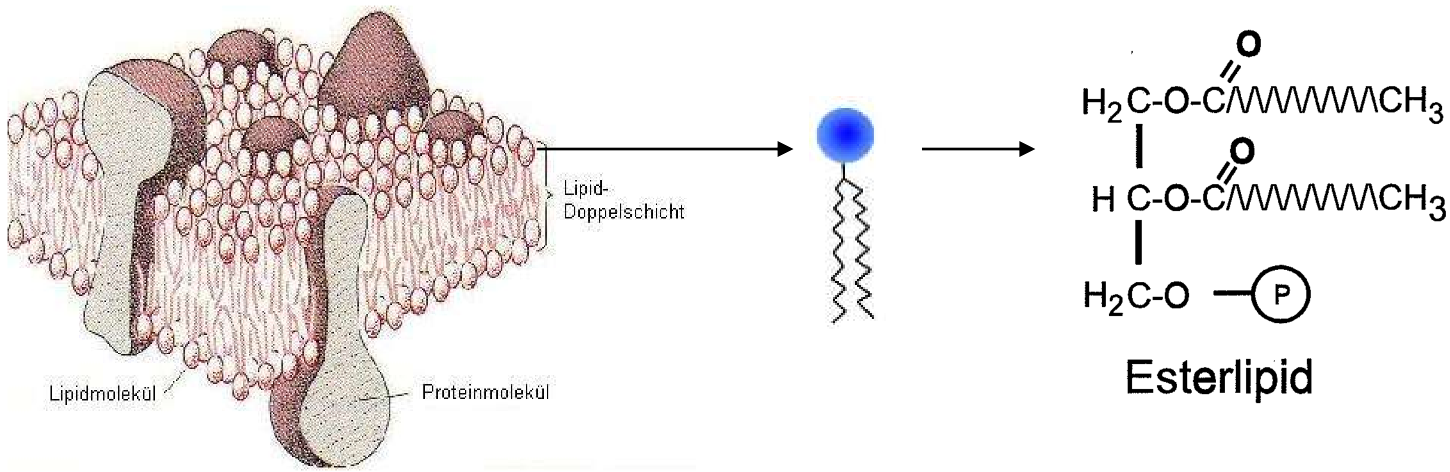

5890
DELTA plus XL

Turnover Time of Soil Pyrolysis Products

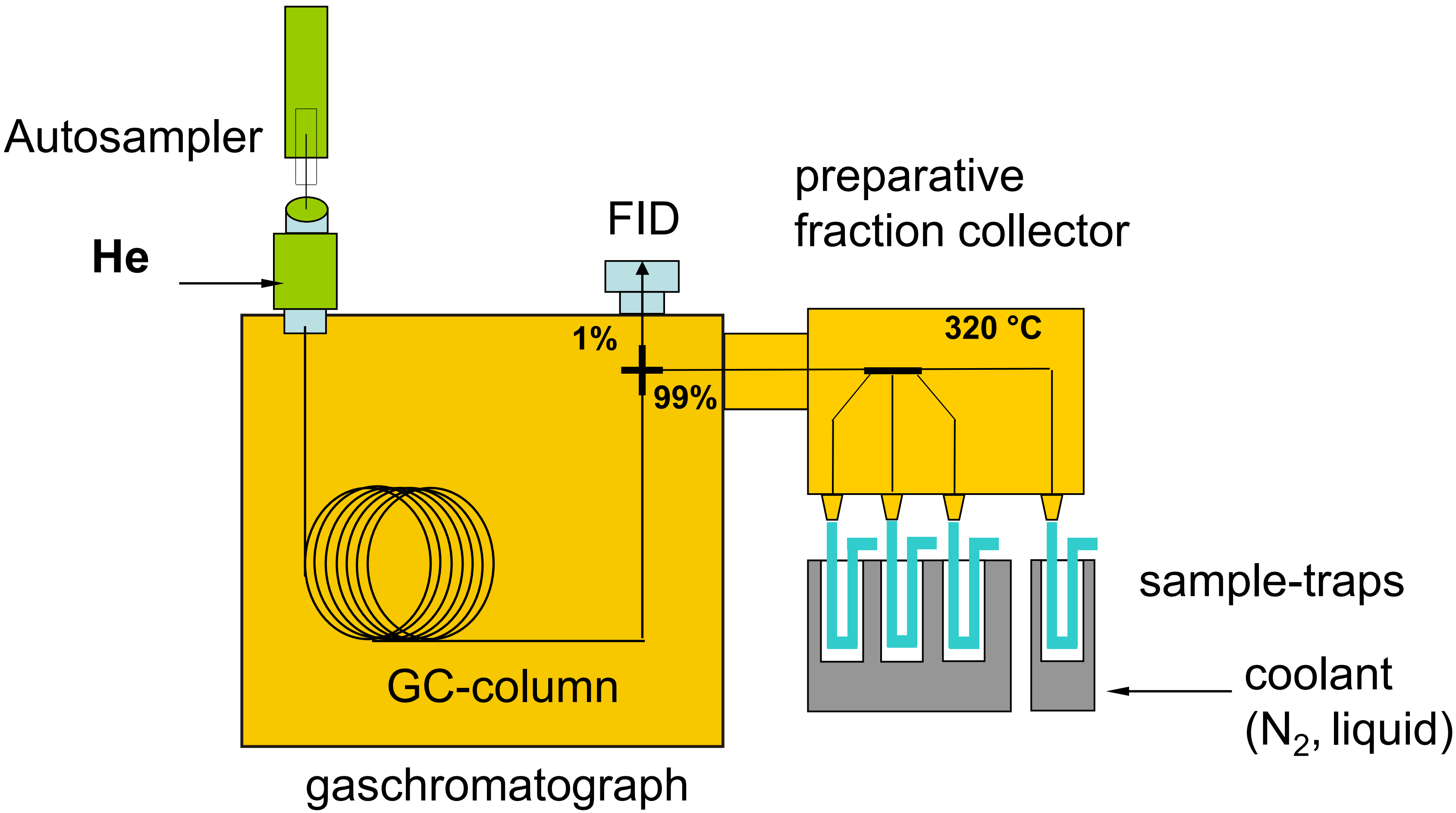


Gleixner et al., Org. Geochem. 2002

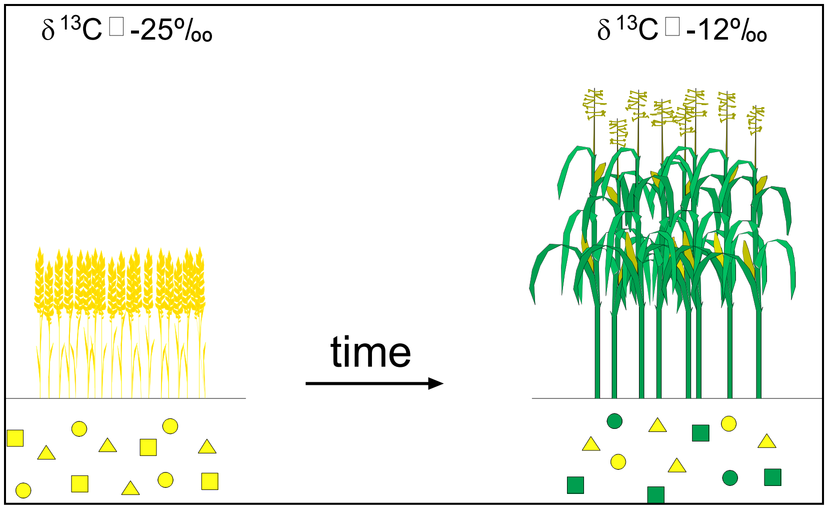
PLFA - Phospholipid fatty acids



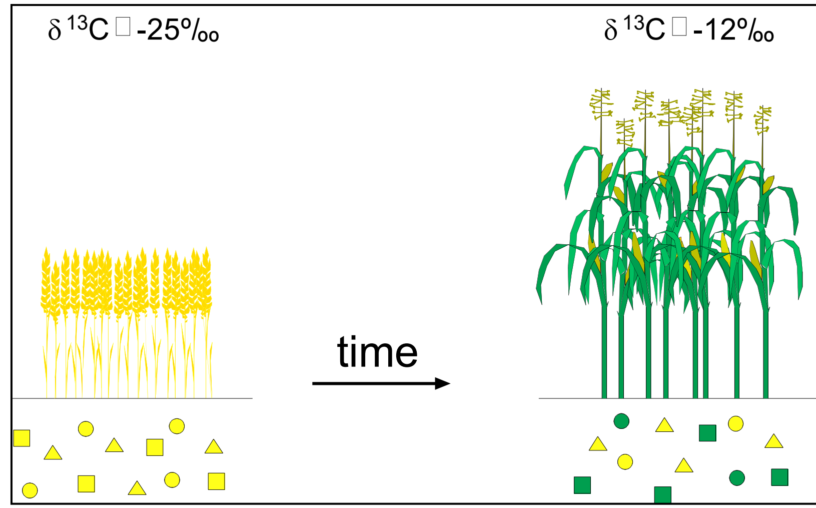
Compound specific ^{14}C ages



Natural Double Labeling Experiment



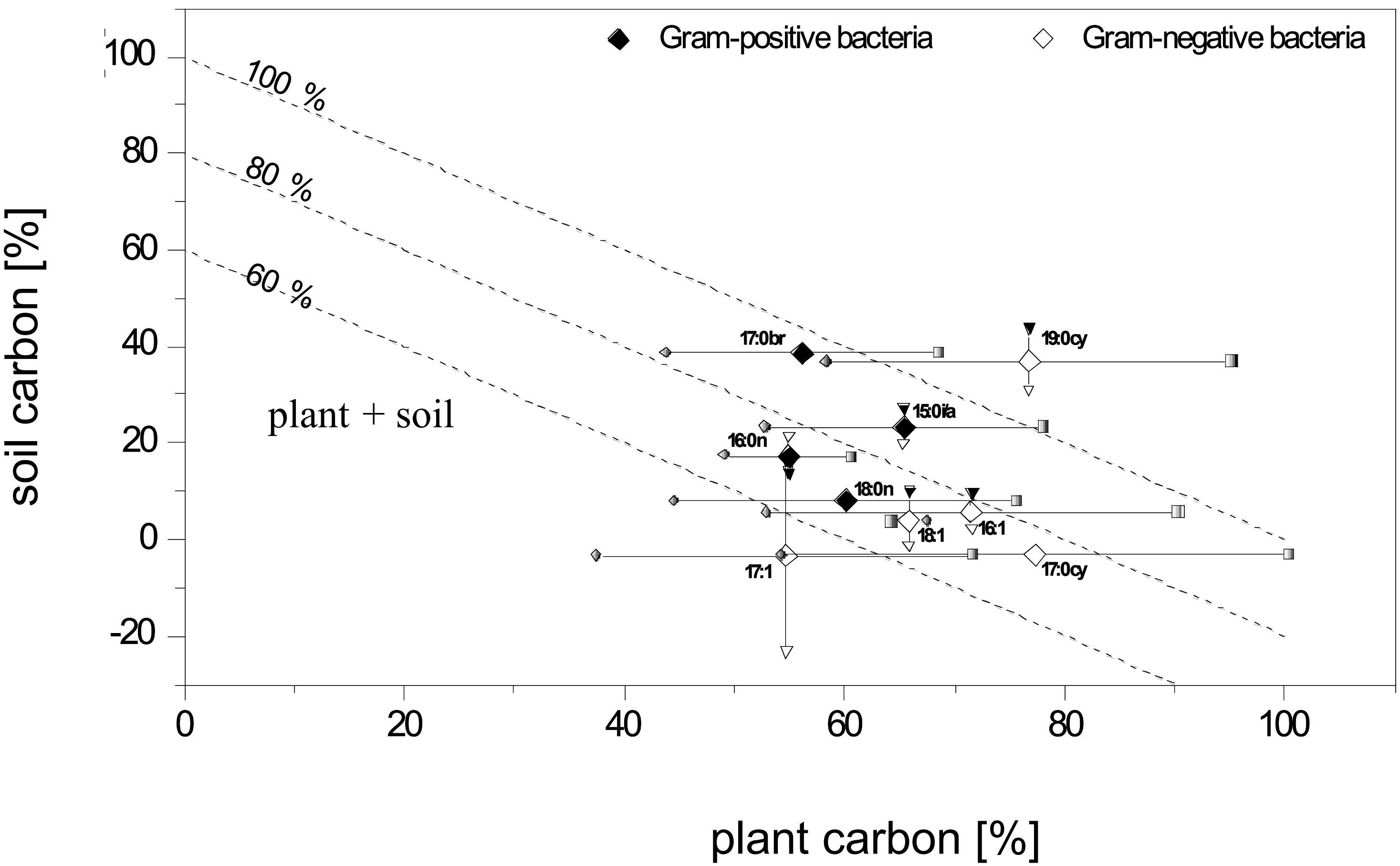
Site 1: High ^{14}C content



Site 2: Low ^{14}C content

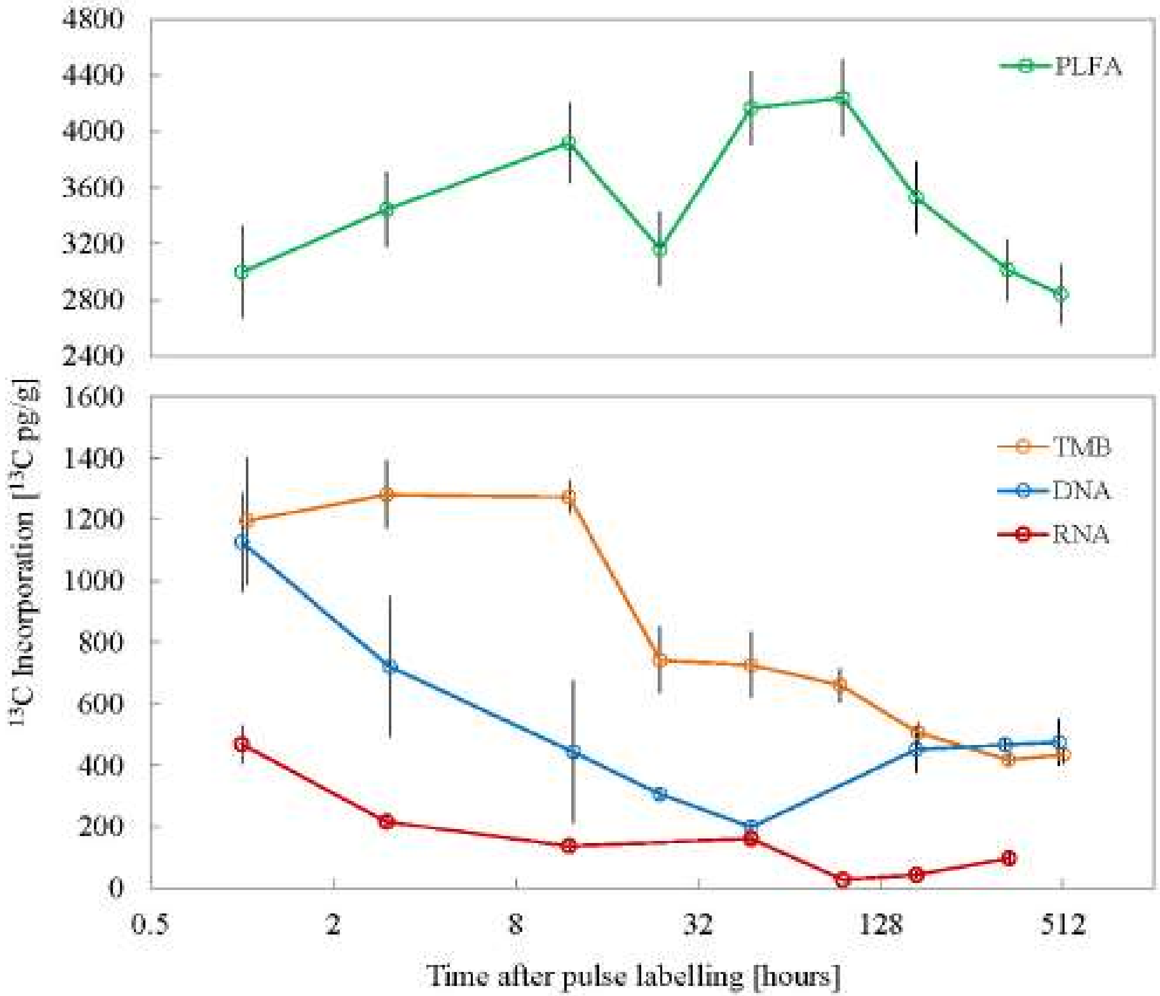
^{13}C indicates plant carbon
^{14}C indicates soil carbon

Carbon sources of microorganisms



Kramer & Gleixner, 2006

Tracing carbon into the microbial community



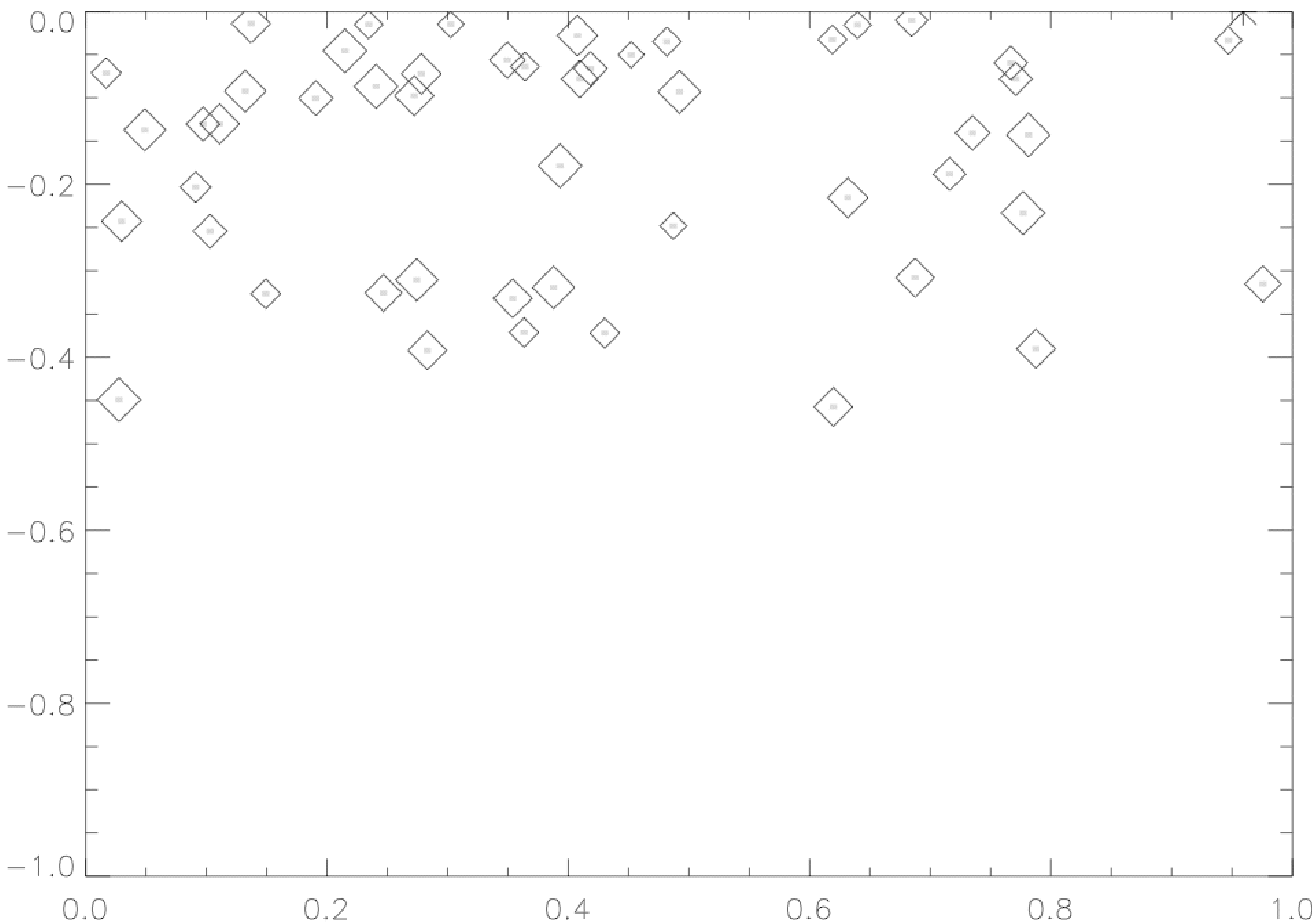
Malik, Front. Microb. 2015

Consequences of living soil

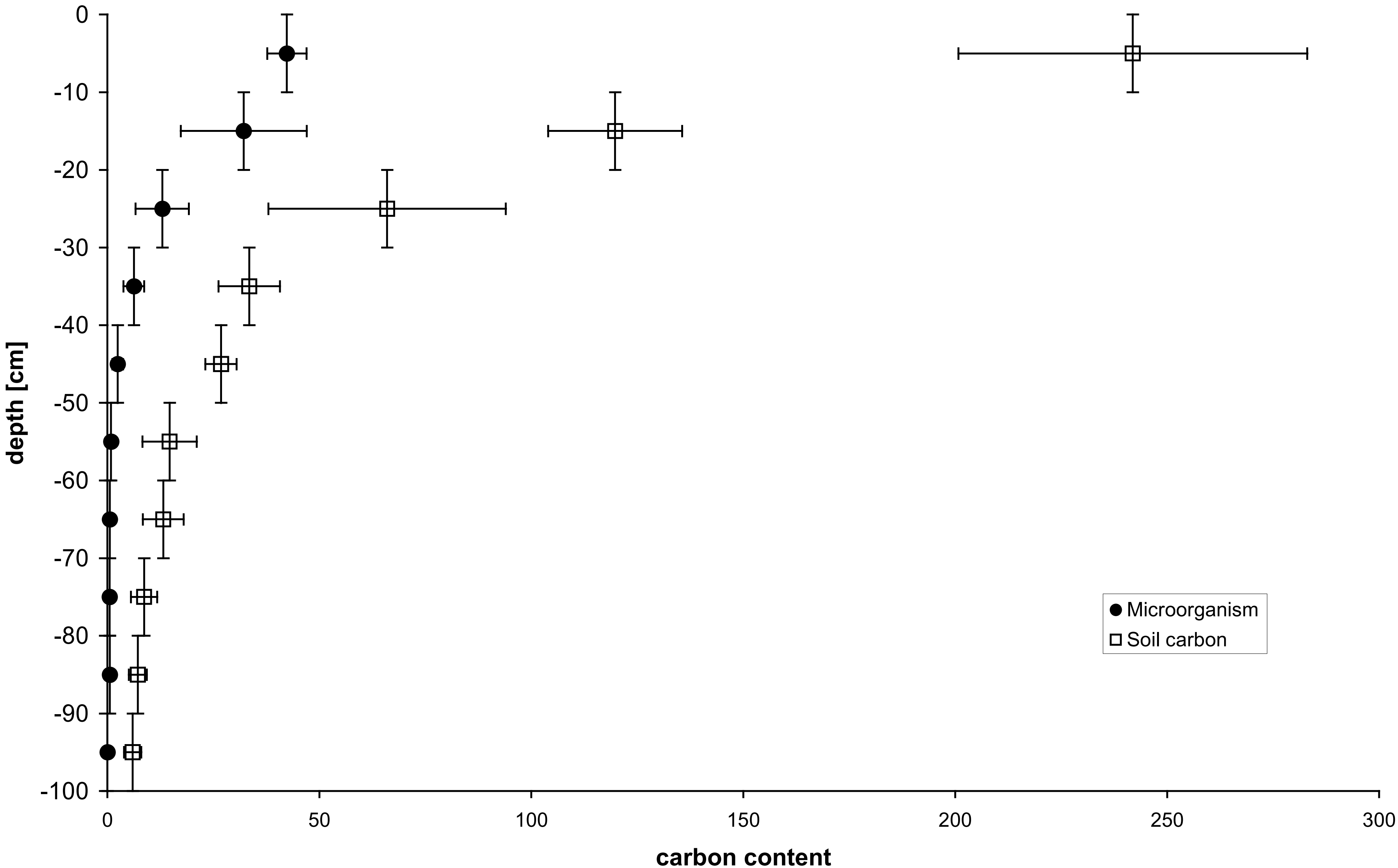
- $C = C_0 e^{kt}$ physical/chemical decay
- $S + E \leftrightarrow ES \rightarrow E + P$ biological decomposition
- $V = V_{max}[S] / K_M + [S]$

Don et al, 2015

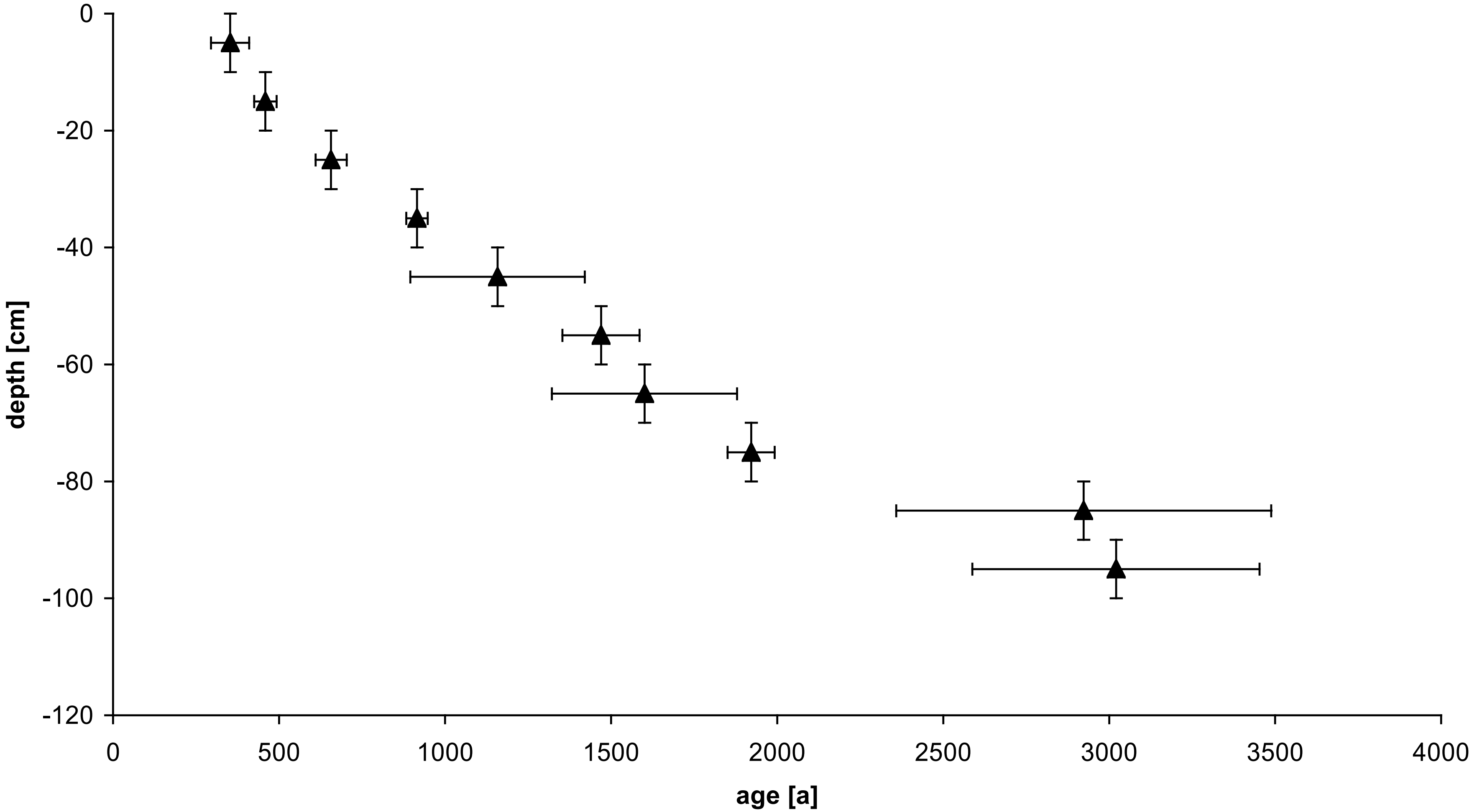
High life in soil



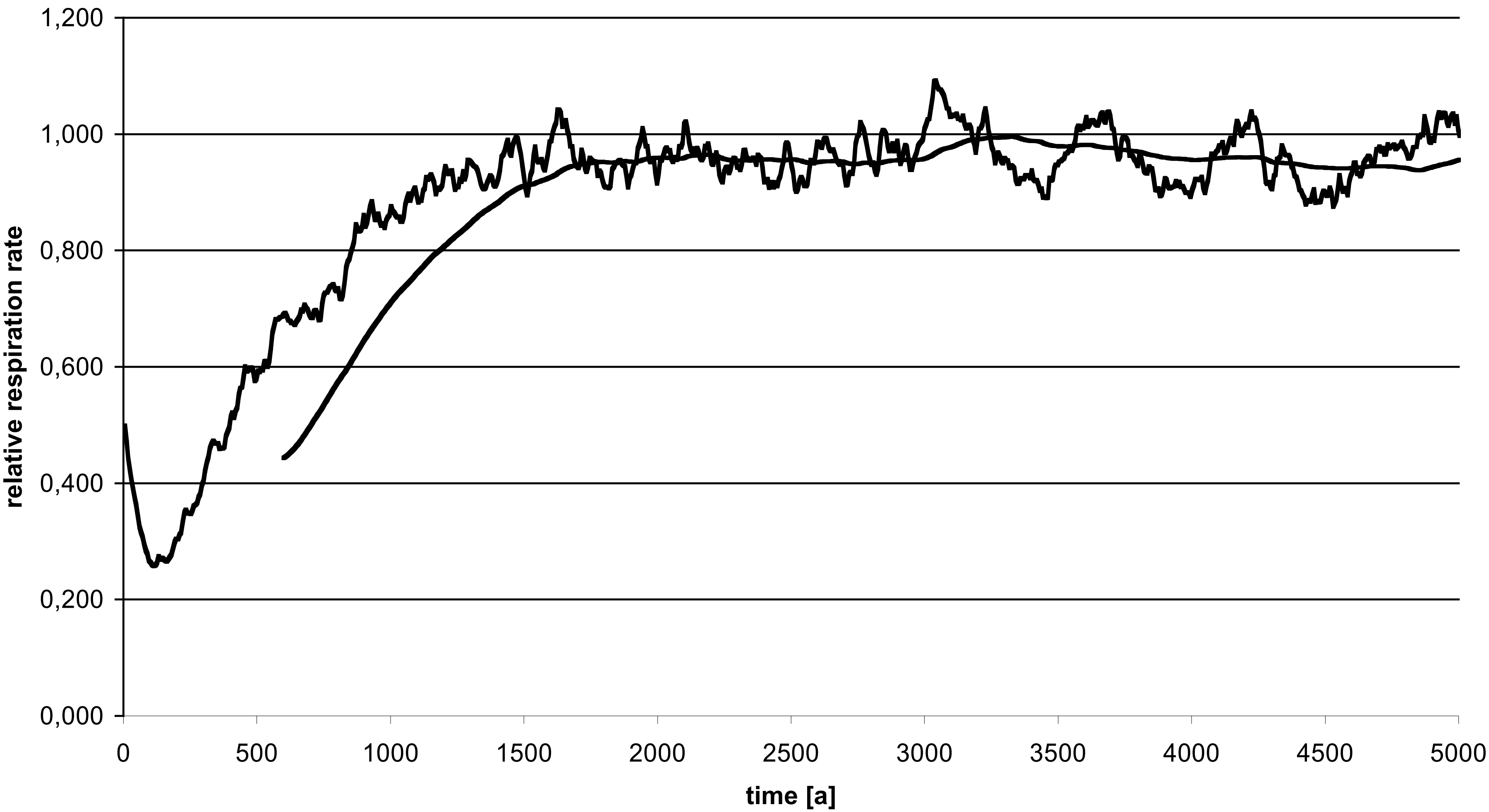
Carbon distribution



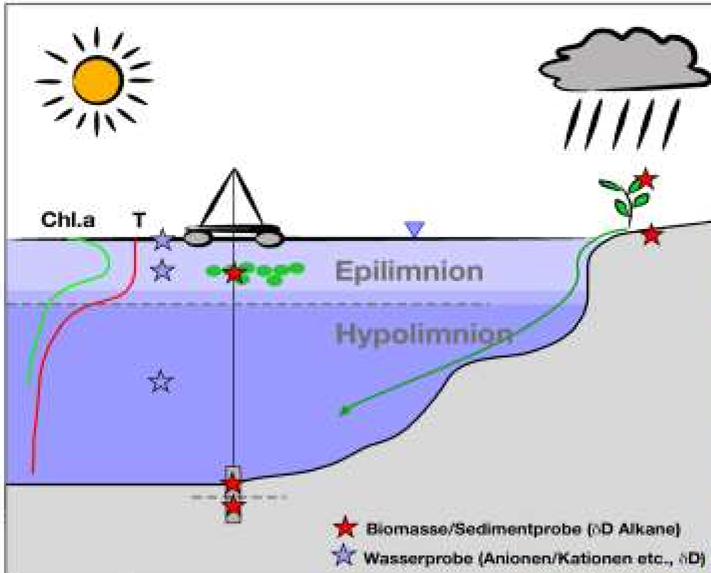
Age distribution



Relative respiration rate



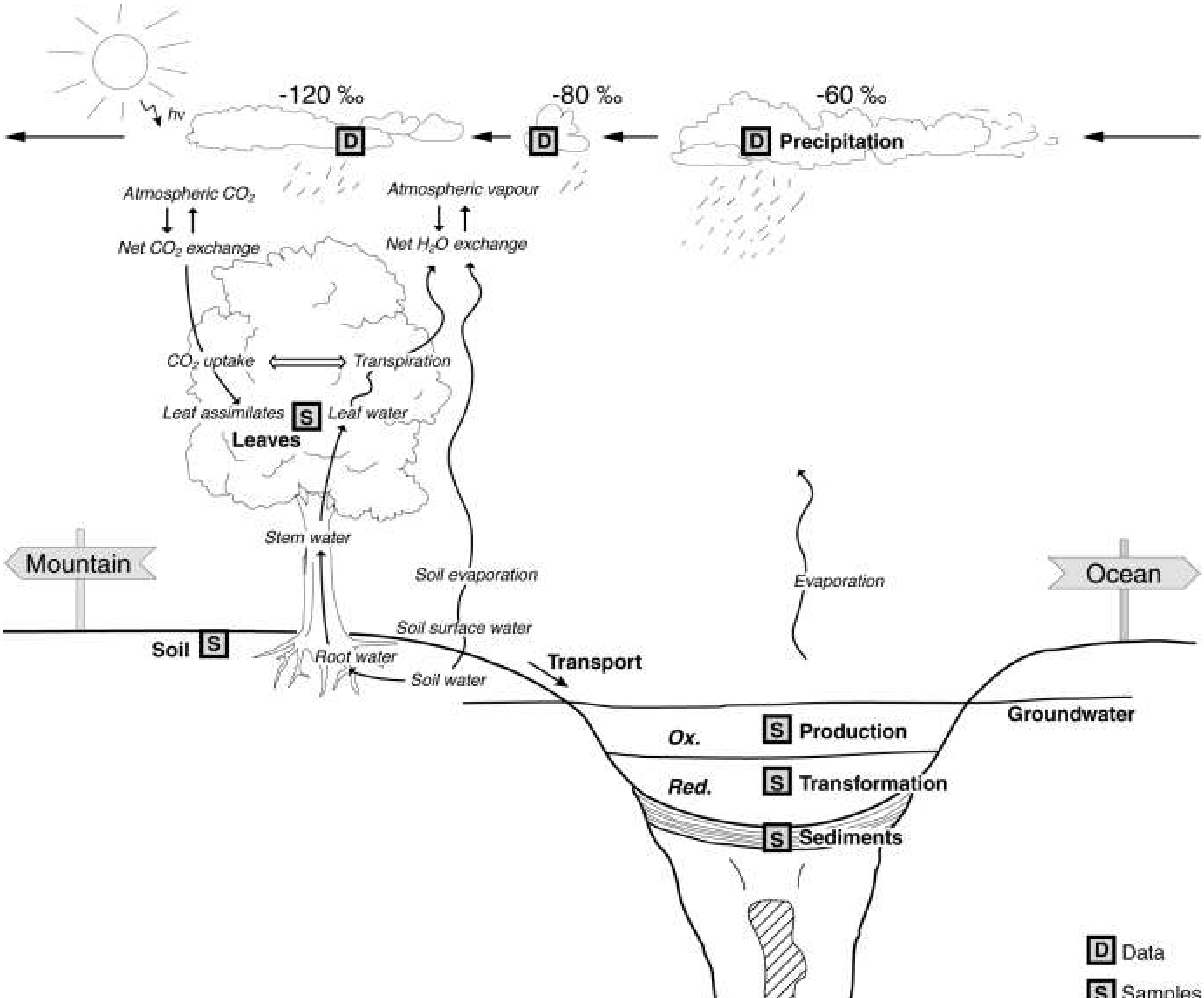
Hydrogen Isotope European Tour 2002



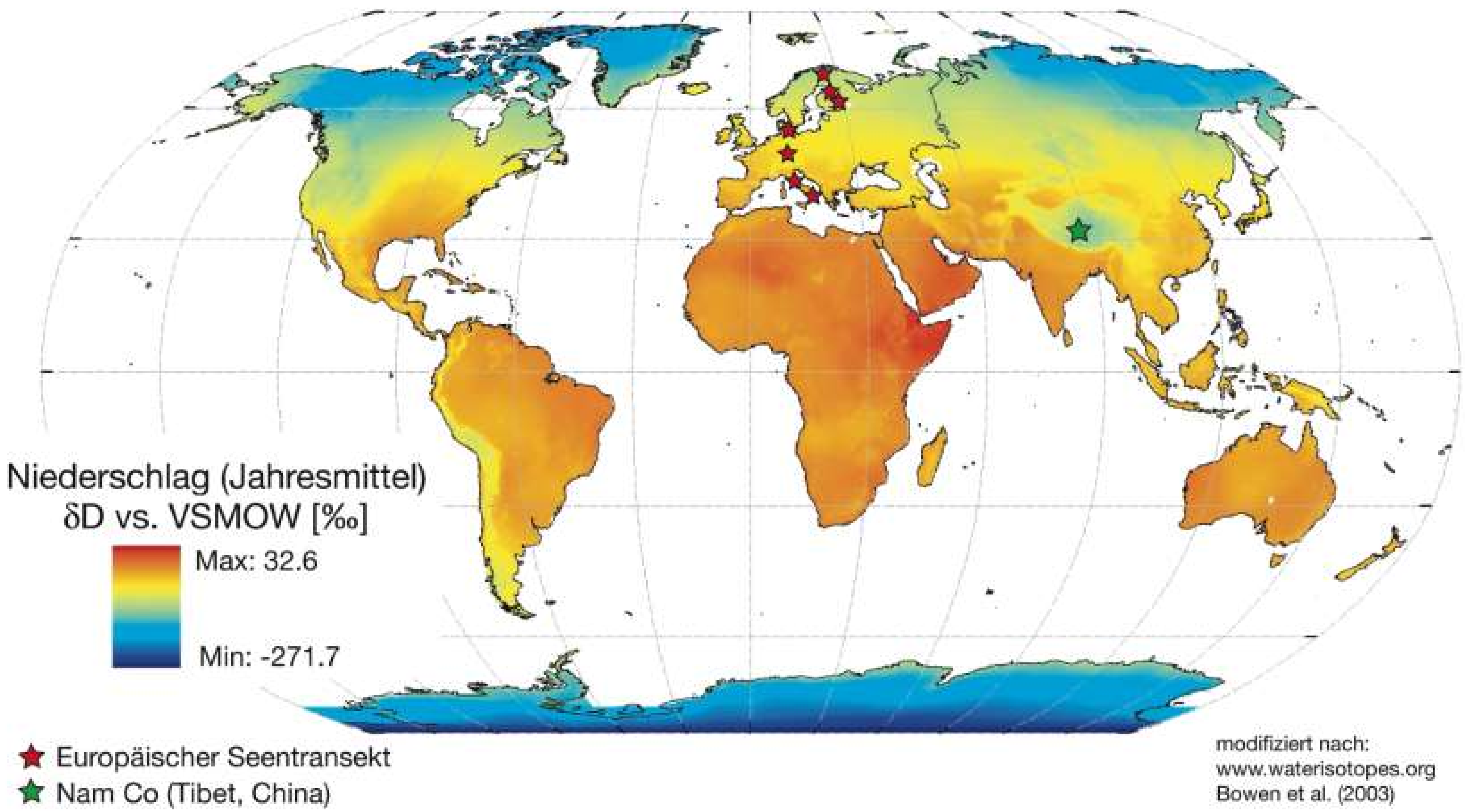
MPI-BGC
Jena

feat. Research Vessel "Tante Käthe"

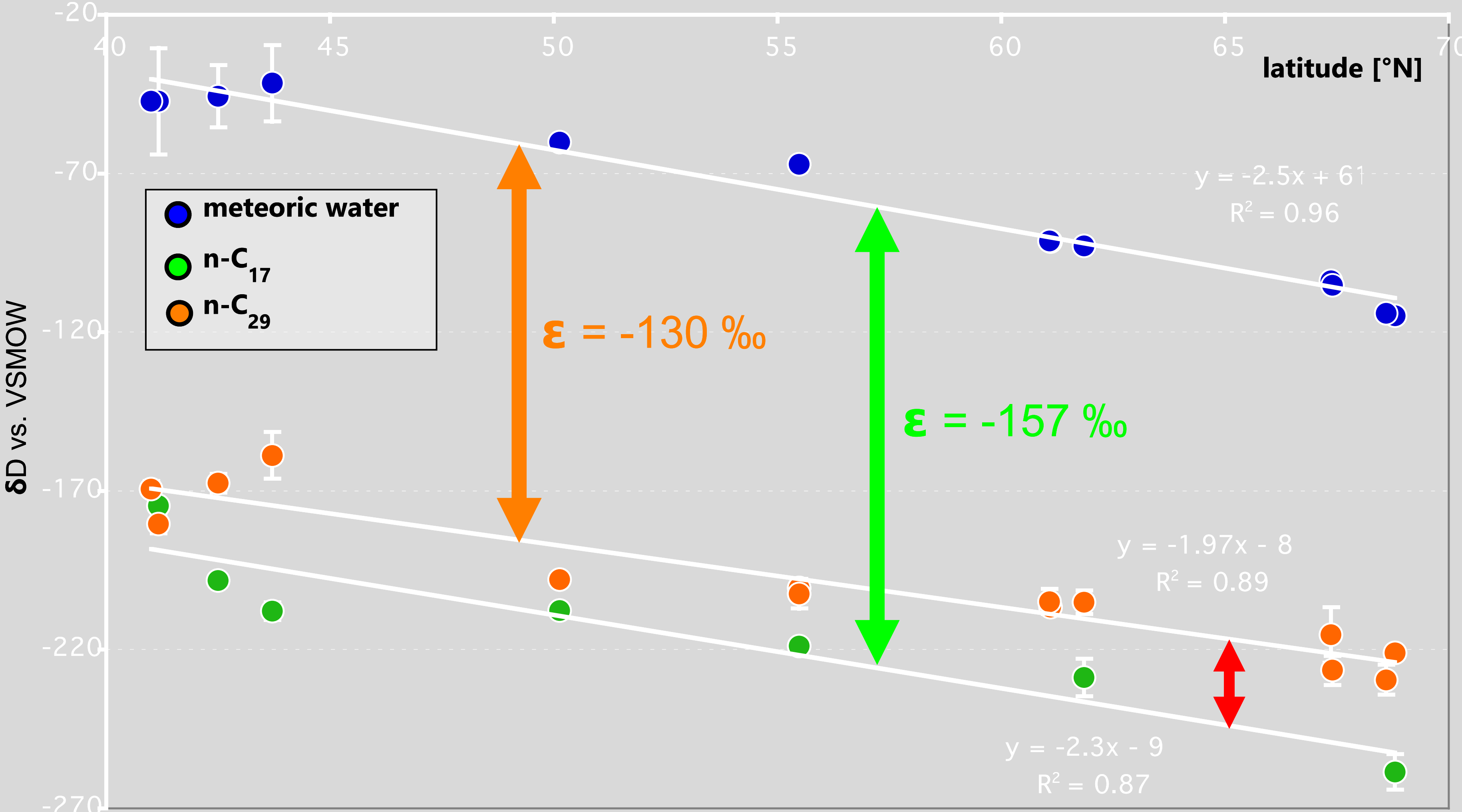
Terrestrial climate archives



Deuterium in Precipitation

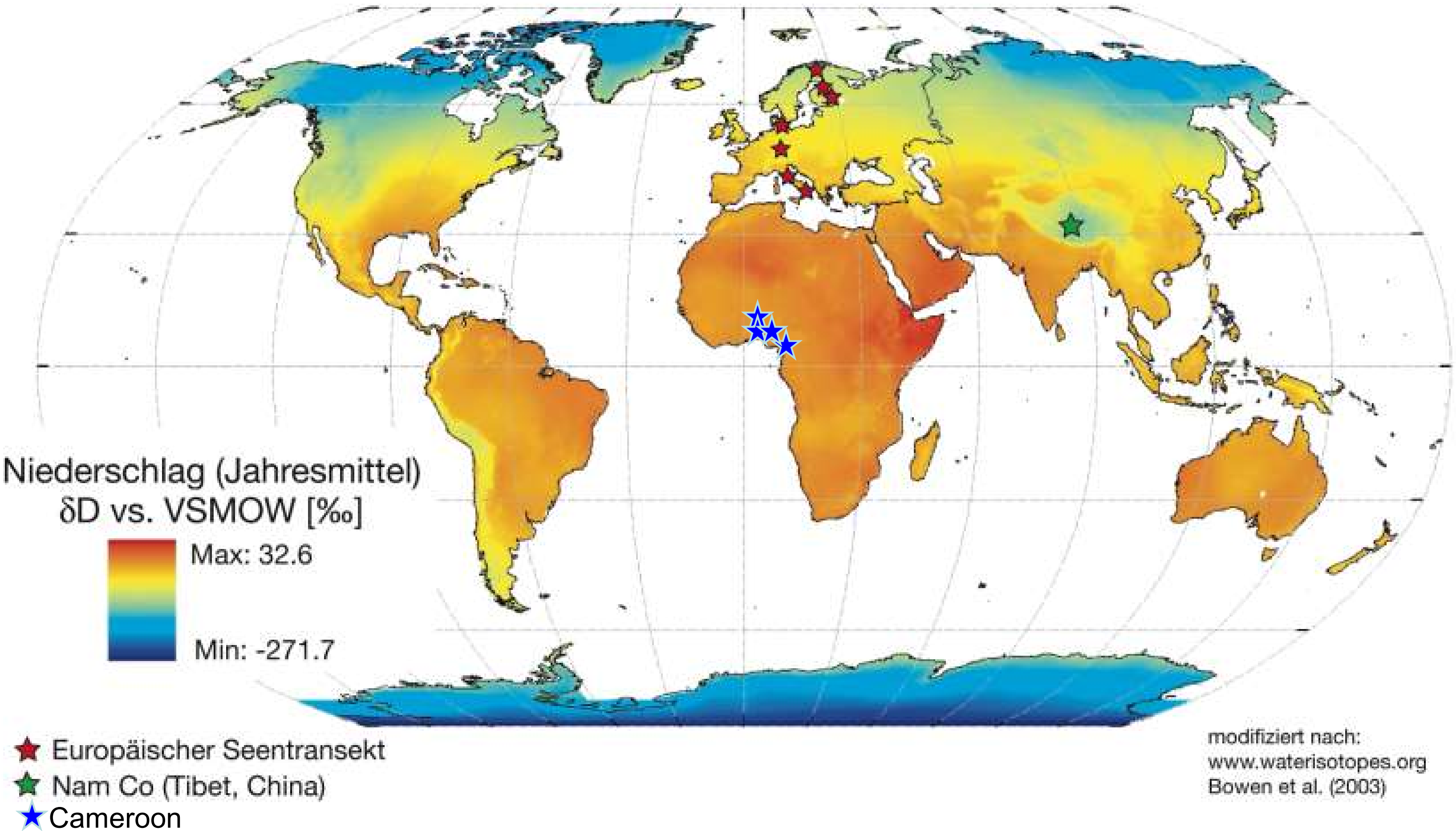


δD values of n-alkanes in modern lake sediments

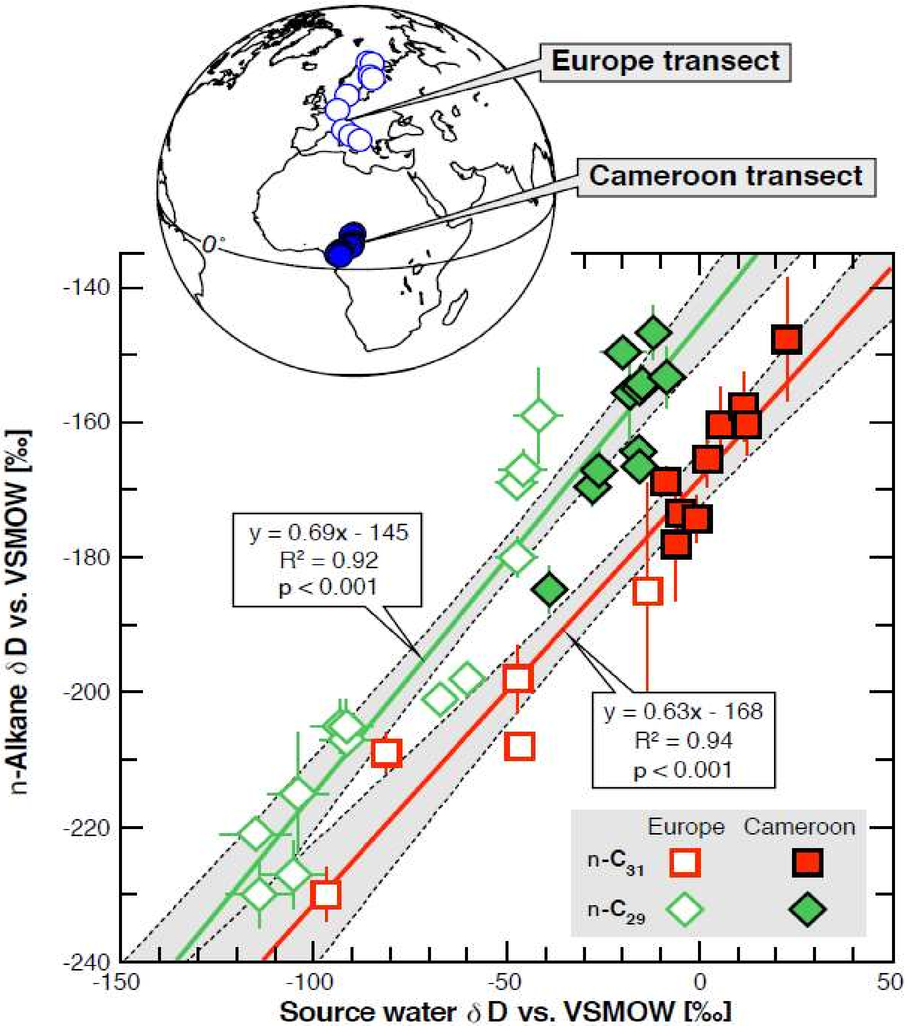


Sachse et al. (2004) *GCA* 68(23)

Deuterium in Precipitation



Extended Calibration

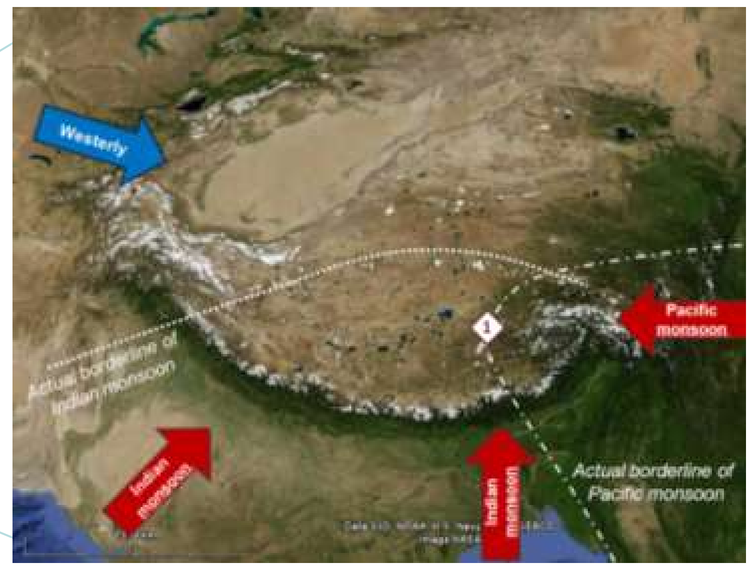


Asian Monsoon



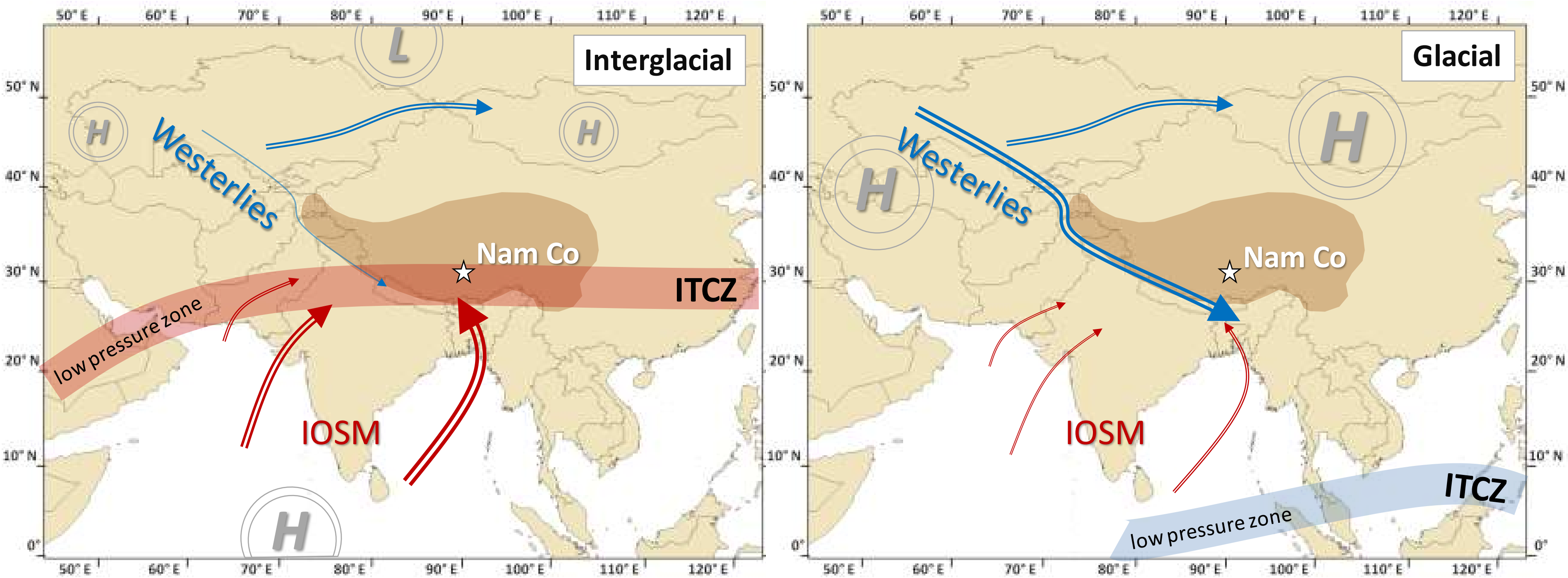






Franziska Günther, Roman Witt, Andrej Thiele, Jeetrendra Saini, Chuanfang Jin

Large Scale Circulation Reconstuction

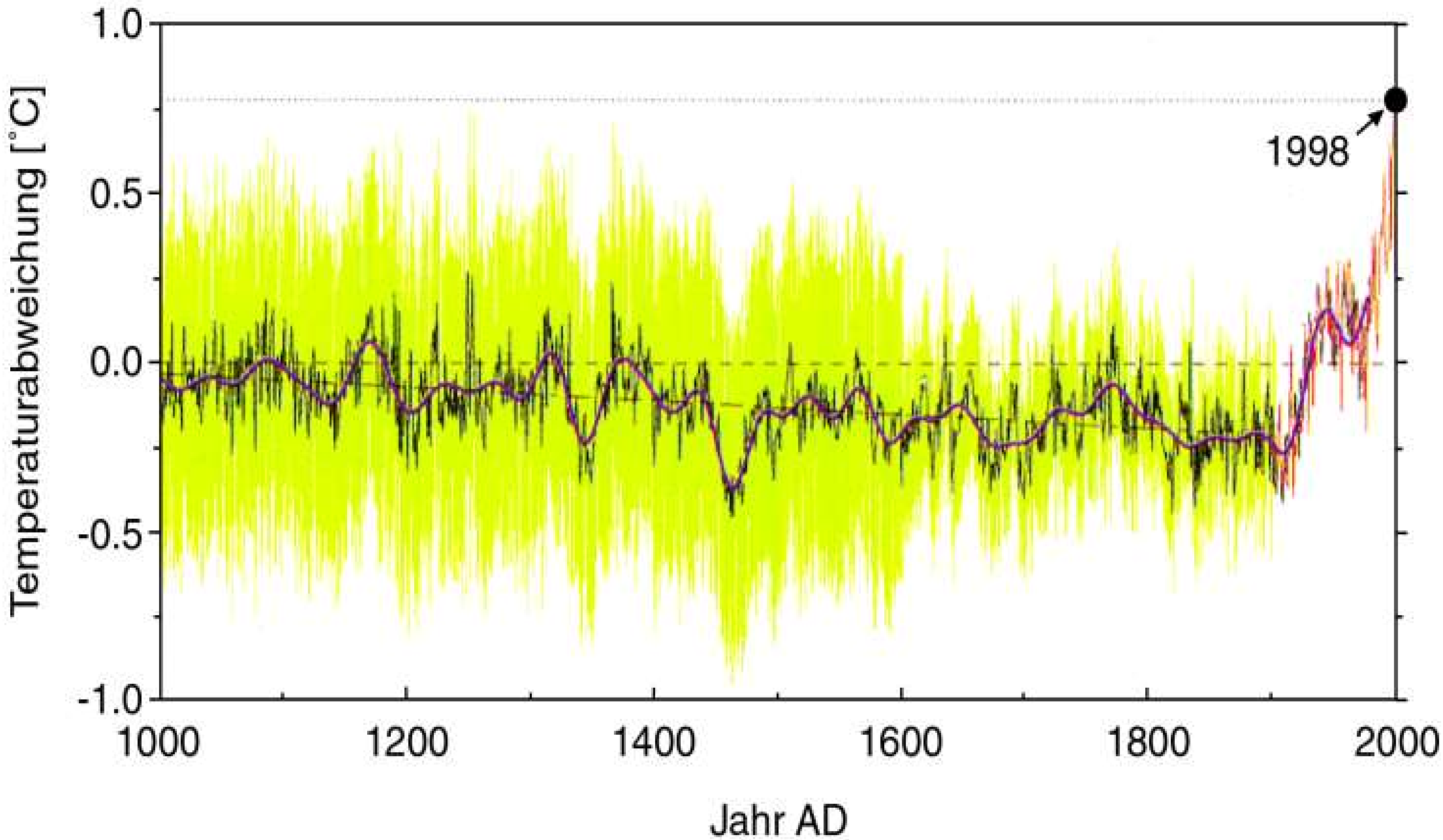


Günther, QSR 2015

Thank you

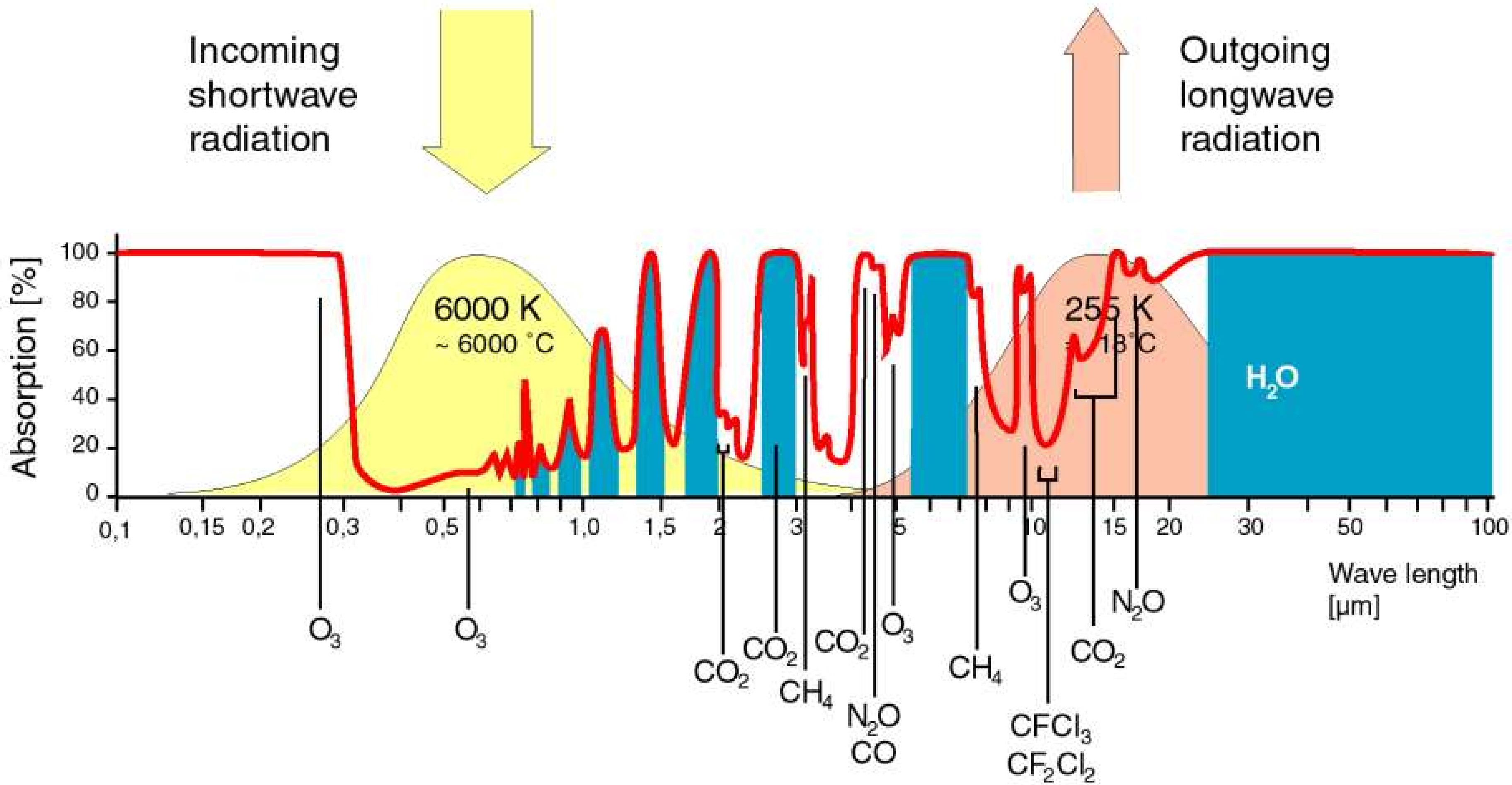
Acknowledgement

- C. Kramer, N. Poirier, A. Börner, S. Grüning,V. Hahn, O. Kracht, B. Lühker, I. Mügler, J. Radke, J. Rothe, S. Rühlow, D. Sachse, S. Steinbeiß, A. Telz, A. Thiele, M. Wengel and M. Werner, MPI Biogeochemistry, Jena
- Zentrale Analytik and Isolab, MPI Biogeochemistry, Jena
- Kate Freeman, Penn State University, USA
- P. Grootes and J. Rethemeyer, Leibnitz Institute, Kiel
- M. Rubino, F. Cotrufo, 2nd University of Naples, I
- R. Bol, Institute of Grassland and Environmental Research, UK
- J. Balesdent, Laboratoire d'Ecologie Microbienne de la Rhizosphere, F
- T. Yao, B. Xu, G. Wu, Inst. Tibetean Plateau Research CAS, CH
- DFG-SPP “Refractory Organic Acids in Water“ and DFG-SPP 1090 “Soils as sink or source for atmospheric CO_2“ for financial support Project Gl 262/1 and Gl 262/4, SPP 1054 “Paläozoic Climate”, FOR 468 “Biodiversity”
- European Commission for a Marie Curie fellowship to N. Poirier
- ESF for travel grant to M. Rubino



Nach Kerr (2000) Science 288:589

Strahlungsschema der Erde

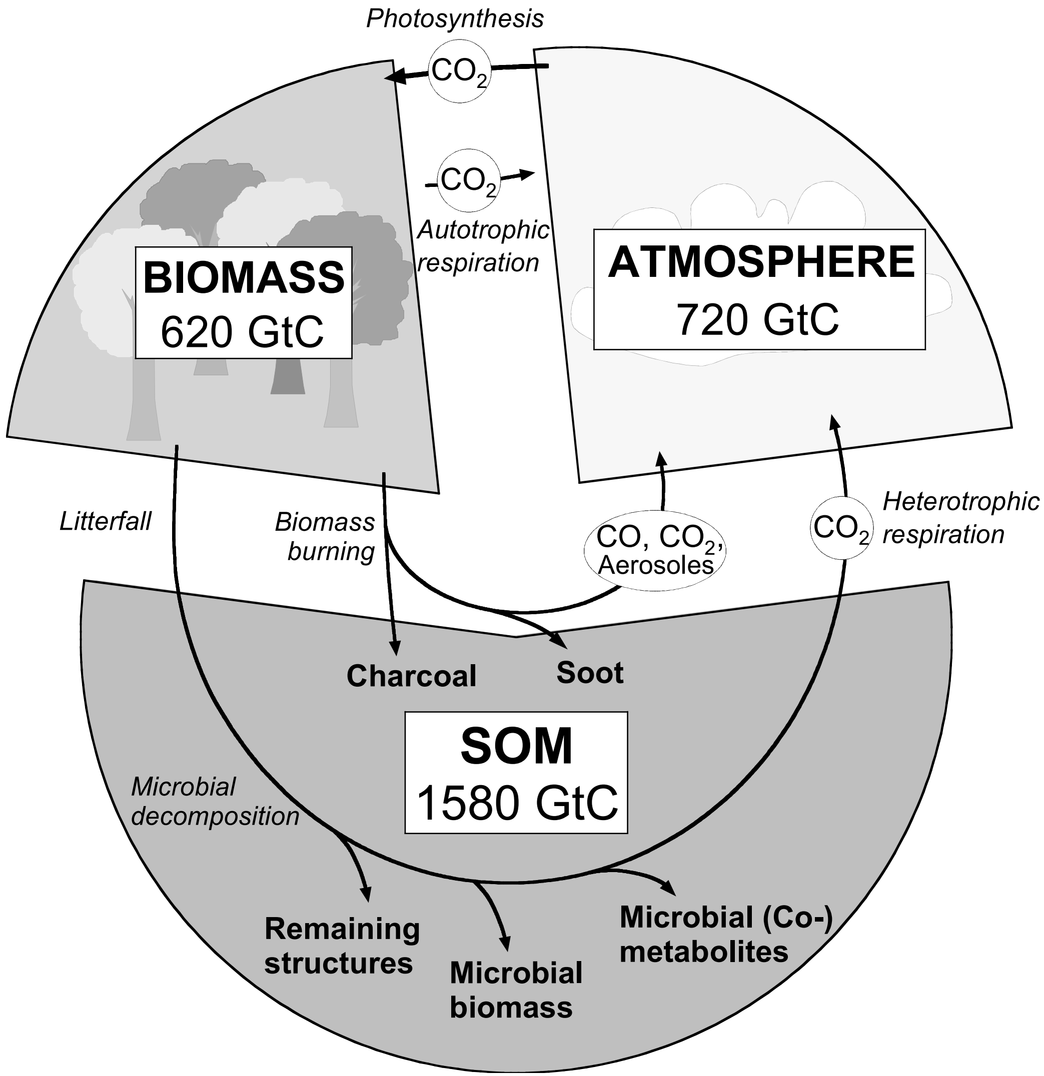


WBGU Bericht, 1988

Greenhouse gases

Gas		Temp. effect [°C]
Water vapour	H_2O	12,8
Carbon dioxide	CO_2	4,4
Ozone	O_3	1,5
Methane	CH_4	0,5
Nitrous oxide	N_2O	0,8

Terrestrial Carbon Cycle

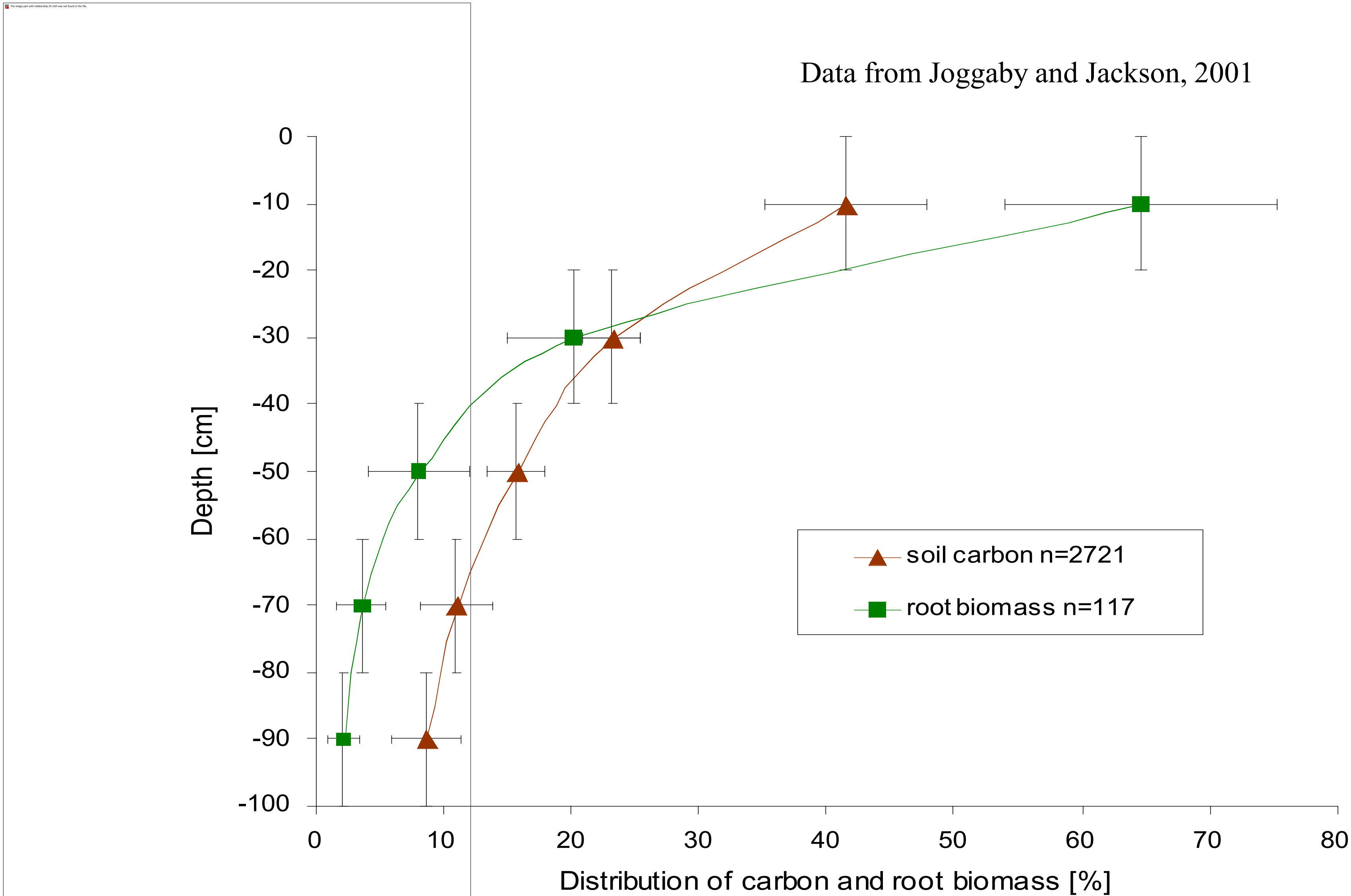


Gleixner et al., 2001

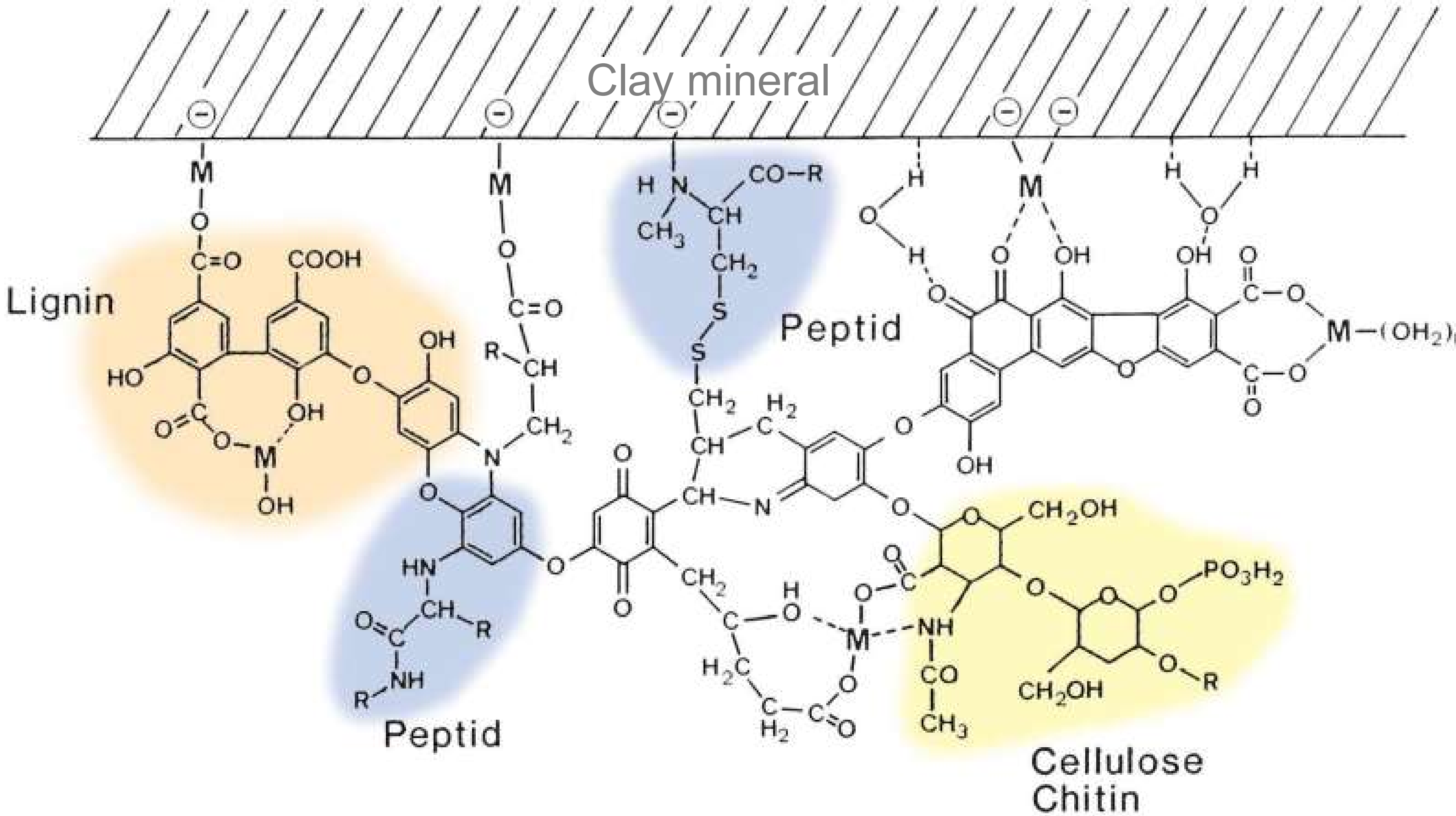
Soil organic matter

- **What we know**
- The role of plant components
- Function of soil organisms
- Conclusions

Soil carbon and roots



Physical Stabilization



after Scheffer, Schachtschabel, 1998

Chemical Stabilization

Biomacromolecules	Occurrence	„Preservation potential“
Cellulose	Vascular plants, some fungi	− / +
Chitin	Arthropods, copepods, crustacea, fungi, algae	+
Lignins	Vascular plants	+ + + +
Tannins	Vascular plants, algae	+ + + / + + + +
Hydrocarbons	Vascular plants, algae	+ + + +
Proteins	All organisms	− / +
Phospholipids	Plants, algae, bacteria	- / +

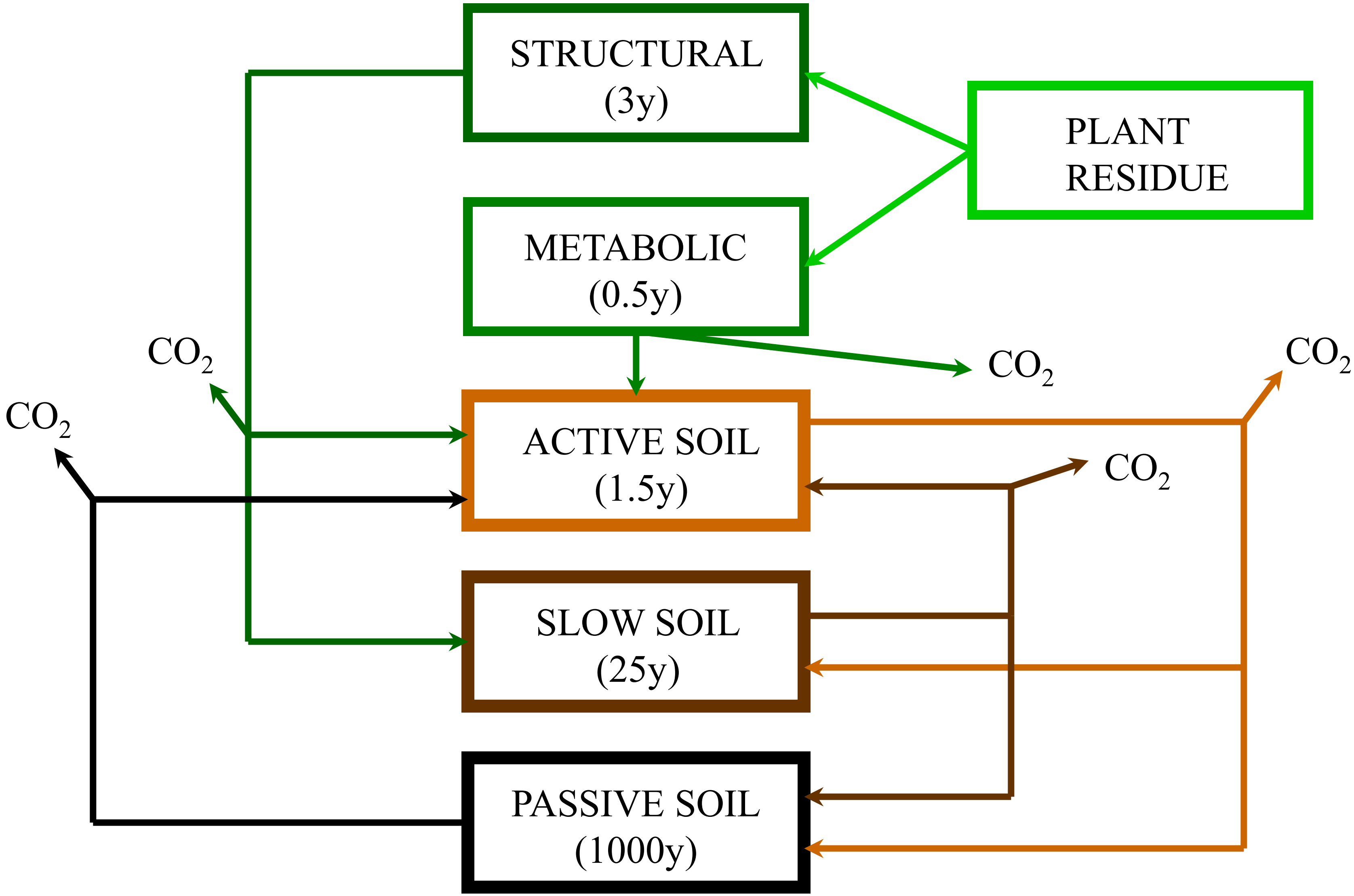
Gleixner et al., 2001

^{14}C Age of Soil Organic Matter

soil		^{14}C age in years (y)	
		top soils	deeper horizonts
peaty gley	British Uplands	modern	10190
podzol	British Uplands	modern	3770
acid brown earth	British Uplands	modern	4630
forest	Brazil	modern	9340
forest	California	386	2193
forest	Midwestern US	422	1712
prairie	Iowa	1072	6272
grasland	Midwestern US	1100	6100
desert	California	19897	21135

Huang et al. 1996, Wang et al. 1996, Pessenda et al. 1996, Paul et al. 2001

Soil carbon models



Stability of soil organic matter

- SOM content is related to input and output
- Stability of SOM is related to chemical and physical properties
- High ^{14}C ages of SOM support the importance of stabilization mechanisms
- Carbon models suggest three pools with annual, decadal and millennium turnover

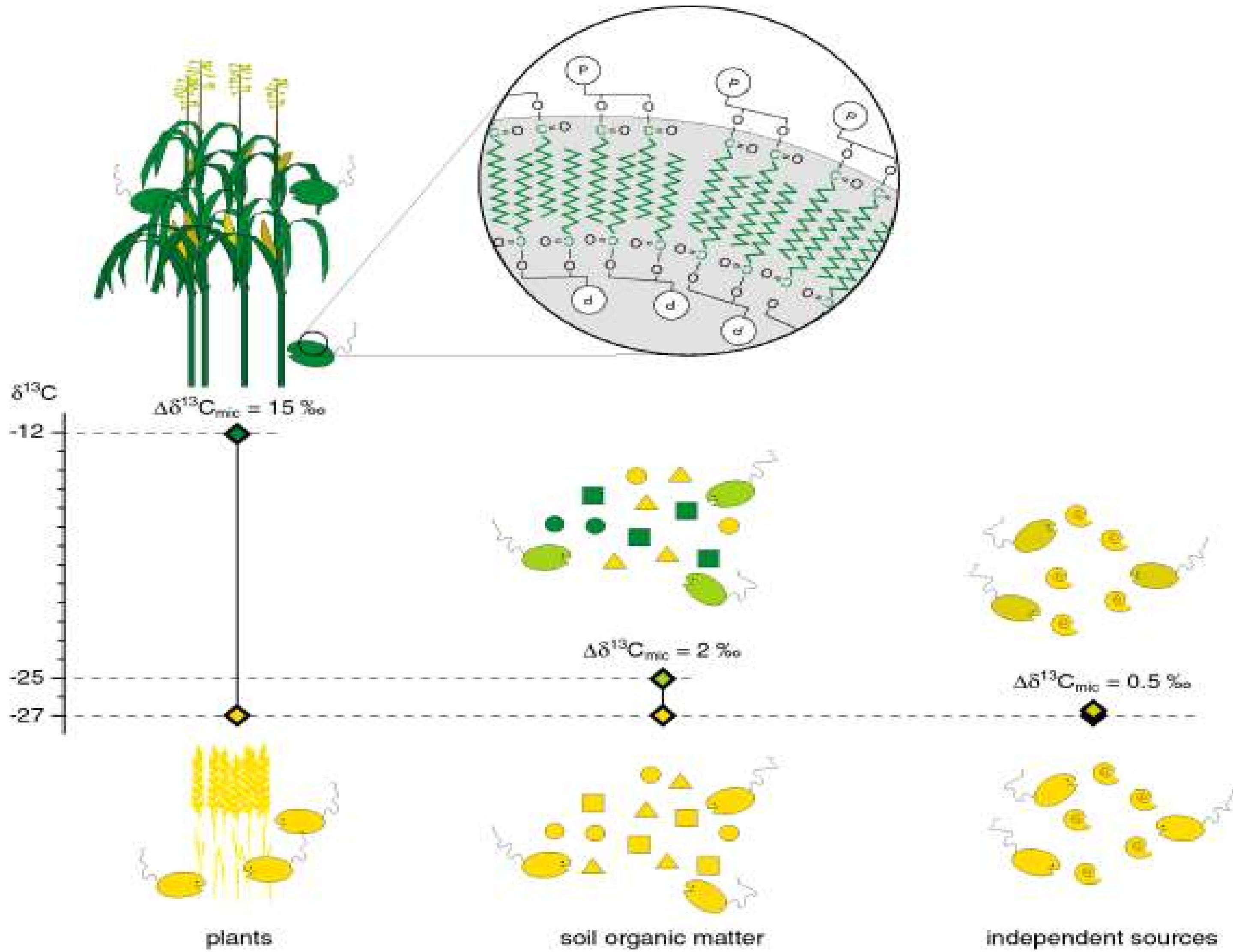
Soil organic matter

- What we know
- **The role of plant components**
- Function of soil organisms
- Conclusion

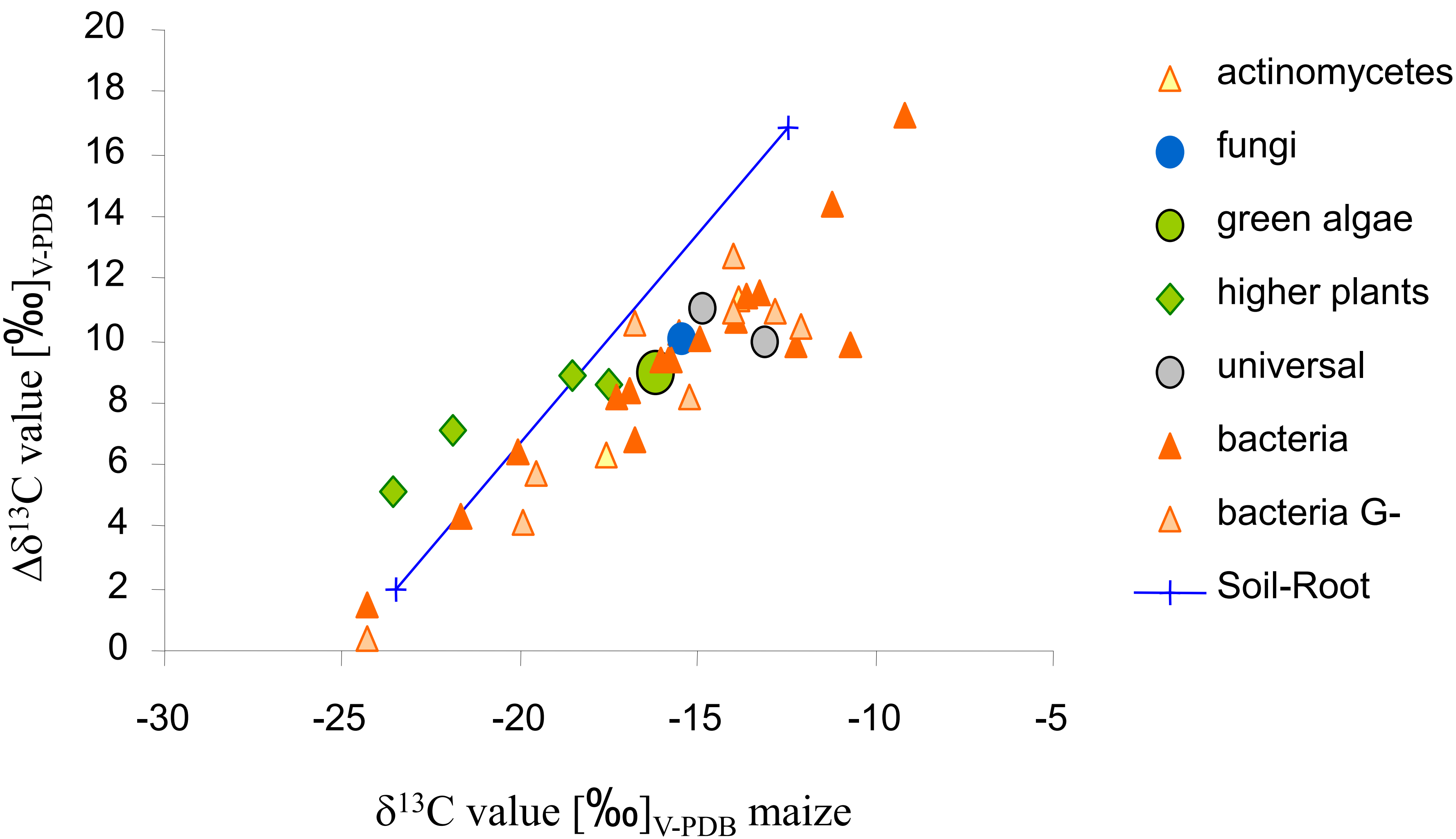
Soil organic matter

- What we know
- The role of plant components
- **Function of soil organisms**
- Conclusion

Microbial Carbon Sources

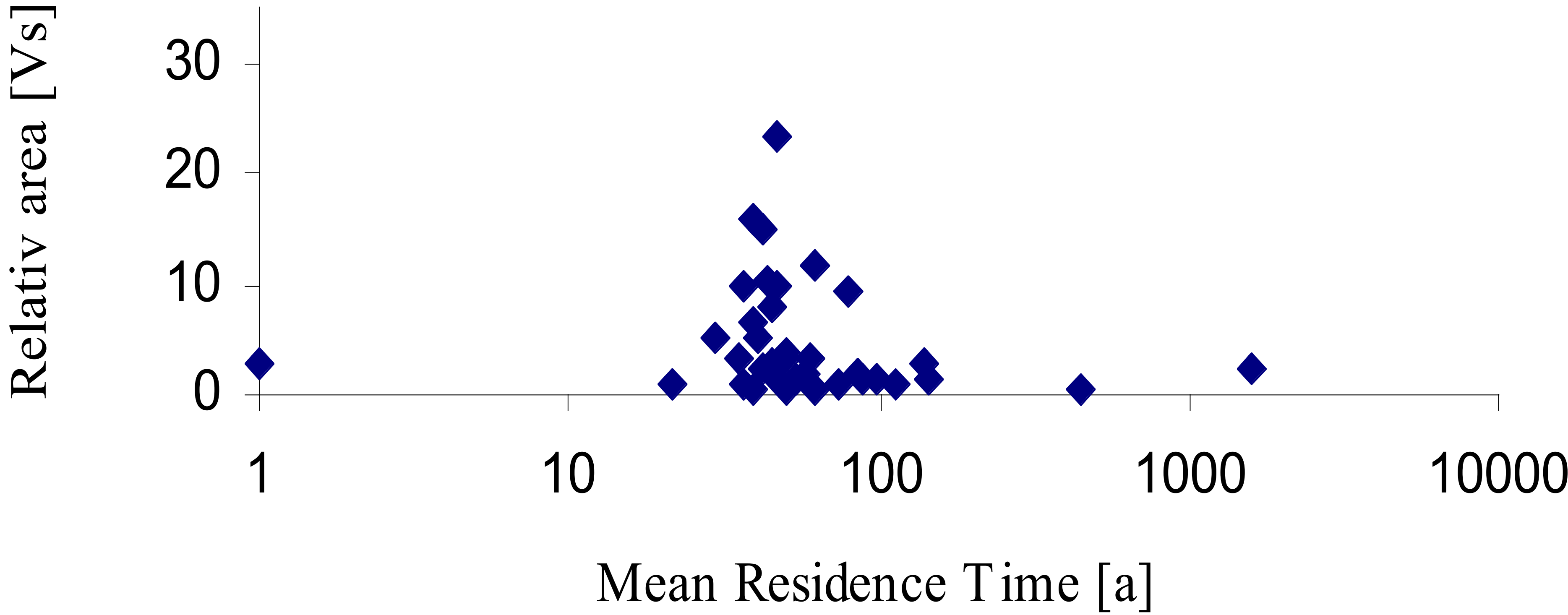


C3 C4 Vegetation Change



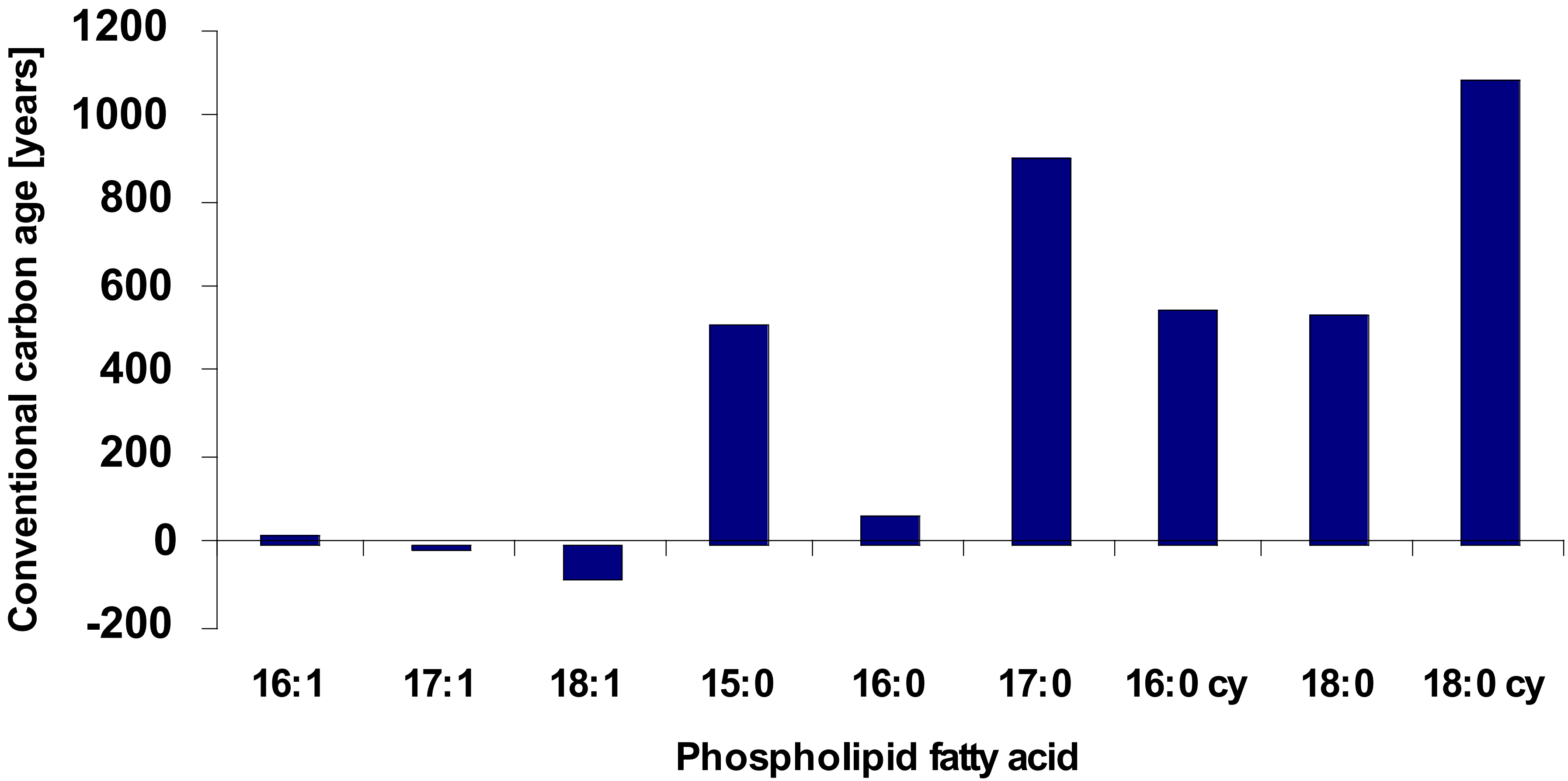
Gleixner et al., 2004

MRT of PLFA



Relativ area [Vs]
30
20
10
0
1
10
100
1000
10000
Mean Residence Time [a]

^{14}C ages of PLFA



Kramer et al., 2004

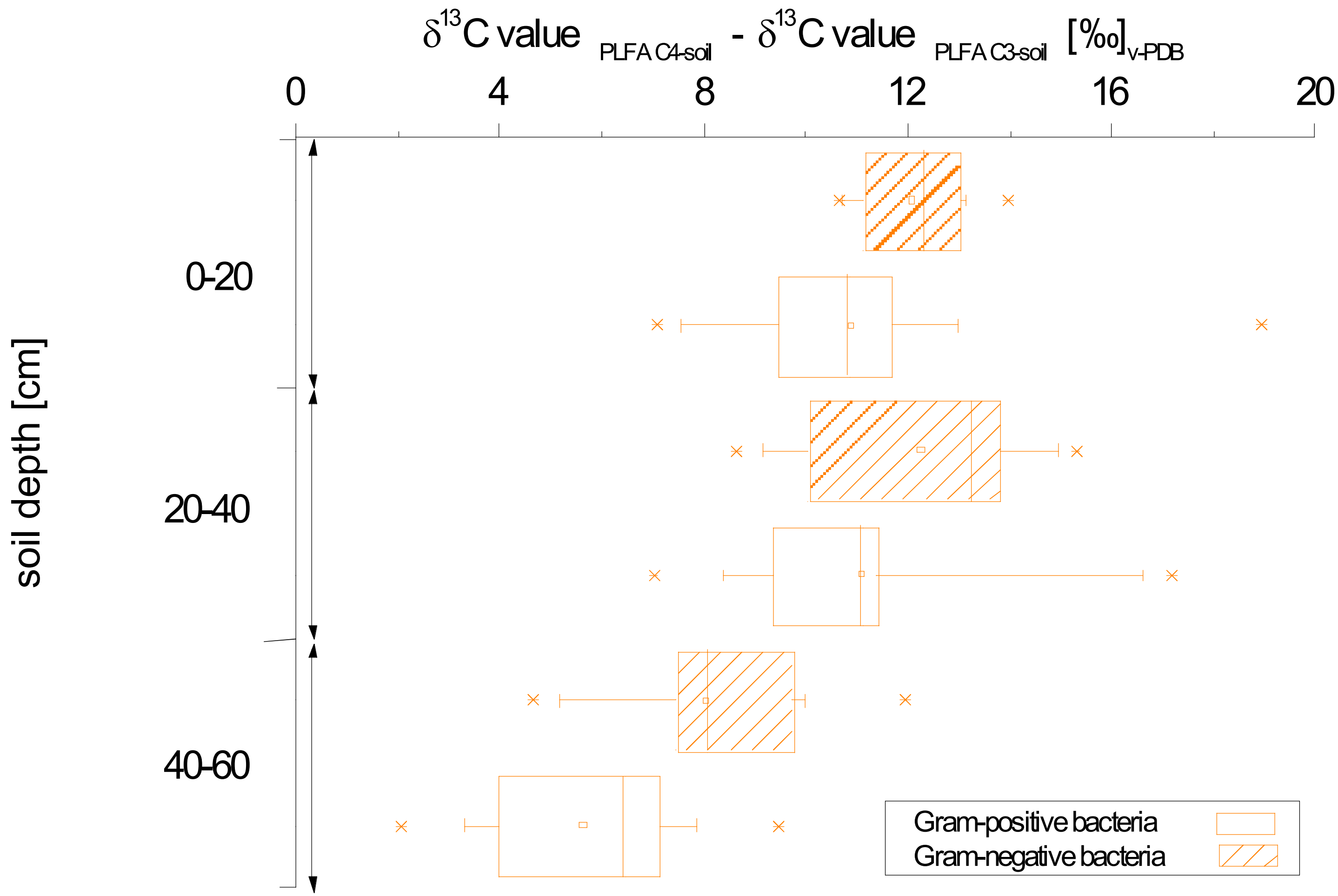
Conclusion

Microbes don’t read books

Soil characteristics of two agricultural sites

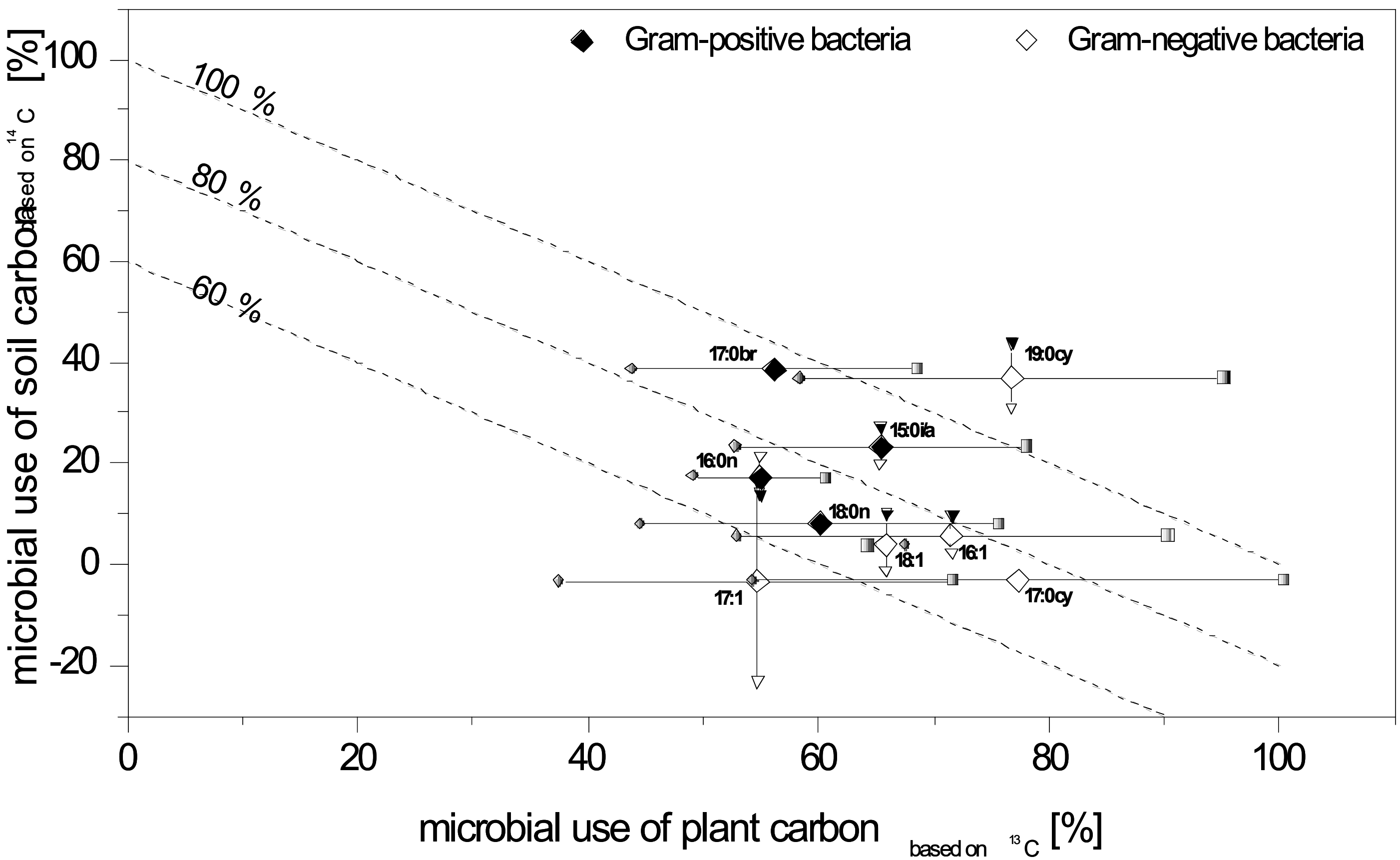
	Halle		*Rotthalmünster*	
Soil type	Haplic Phaeozem		Haplic Luvisol	
Bulk-SOM				
C-content [%]	**1.3**		**1.4**	
N-content [%]	**0.15**		**0.075**	
pMC	**54**		**107**	
pMC of SOM-Fractions				
humin / humic acid	**30 / 47**		**106 / 102**	
total lipids	**46**		**103**	
phospholipids	**58**		**107**	
Density fractions	**yield OC [%]**	**pMC**	**yield OC [%]**	**pMC**
fPOM	**~ 8**	**~ 47**	**~ 3**	**~ 104**
$oPOM_{<1.6}$	**~ 3**	**~ 8**	**~ 0.5**	**~ 98**
$oPOM_{1.6-2.0}$	**~ 16**	**~ 25**	**~ 10**	**~ 104**
mineral	**~ 62**	**~ 59**	**~ 87**	**~ 104**

John.et al. (2005); Kramer (2004); Rethemeyer et al.; (2005)



δ13C value PLFA C4-soil - δ13C value PLFA C3-soil [‰]V-PDB
0
4
8
12
16
20
soil depth [cm]
0-20
20-40
40-60
Gram-positive bacteria
Gram-negative bacteria

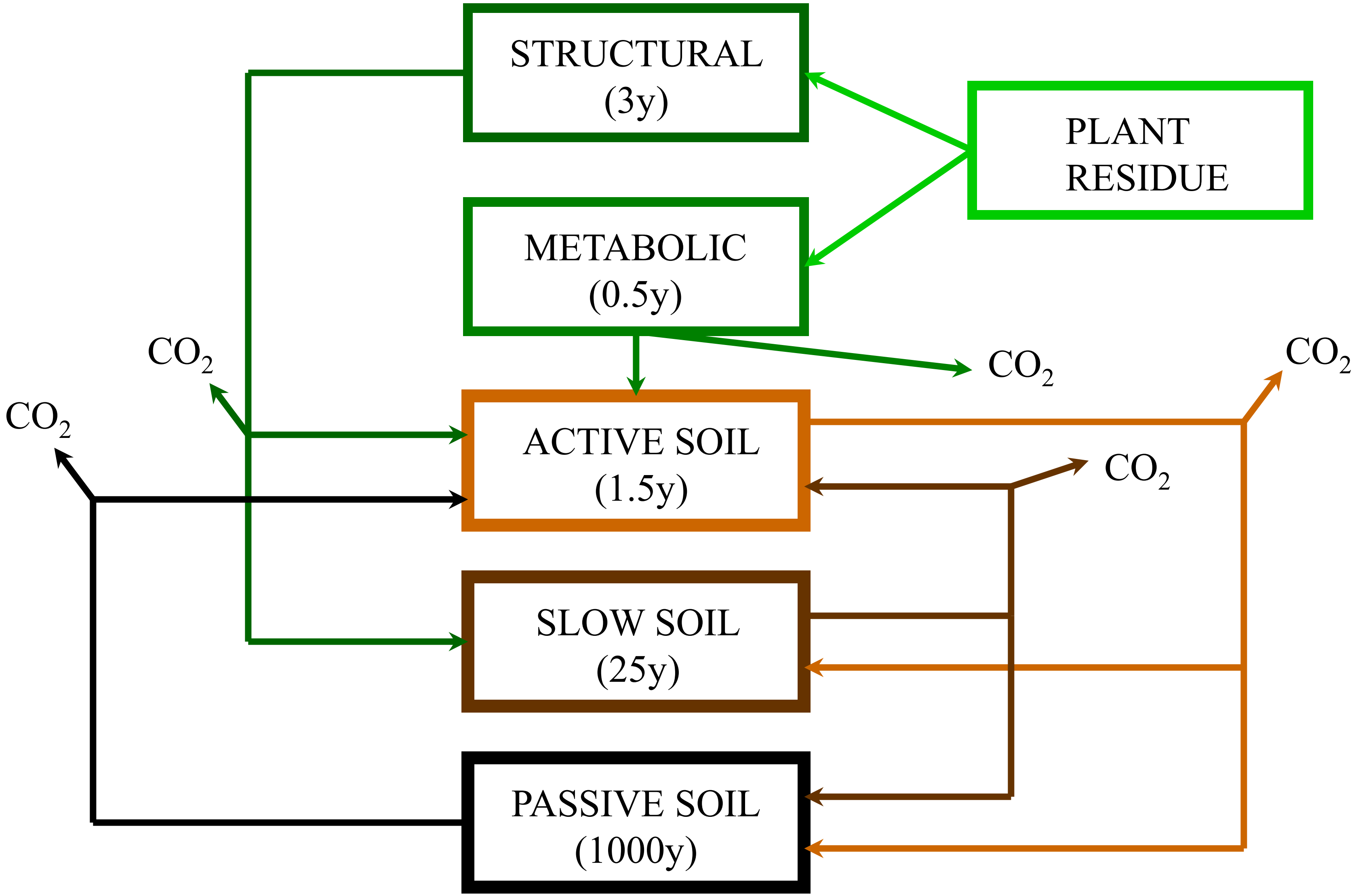
Carbon sources of microorganisms

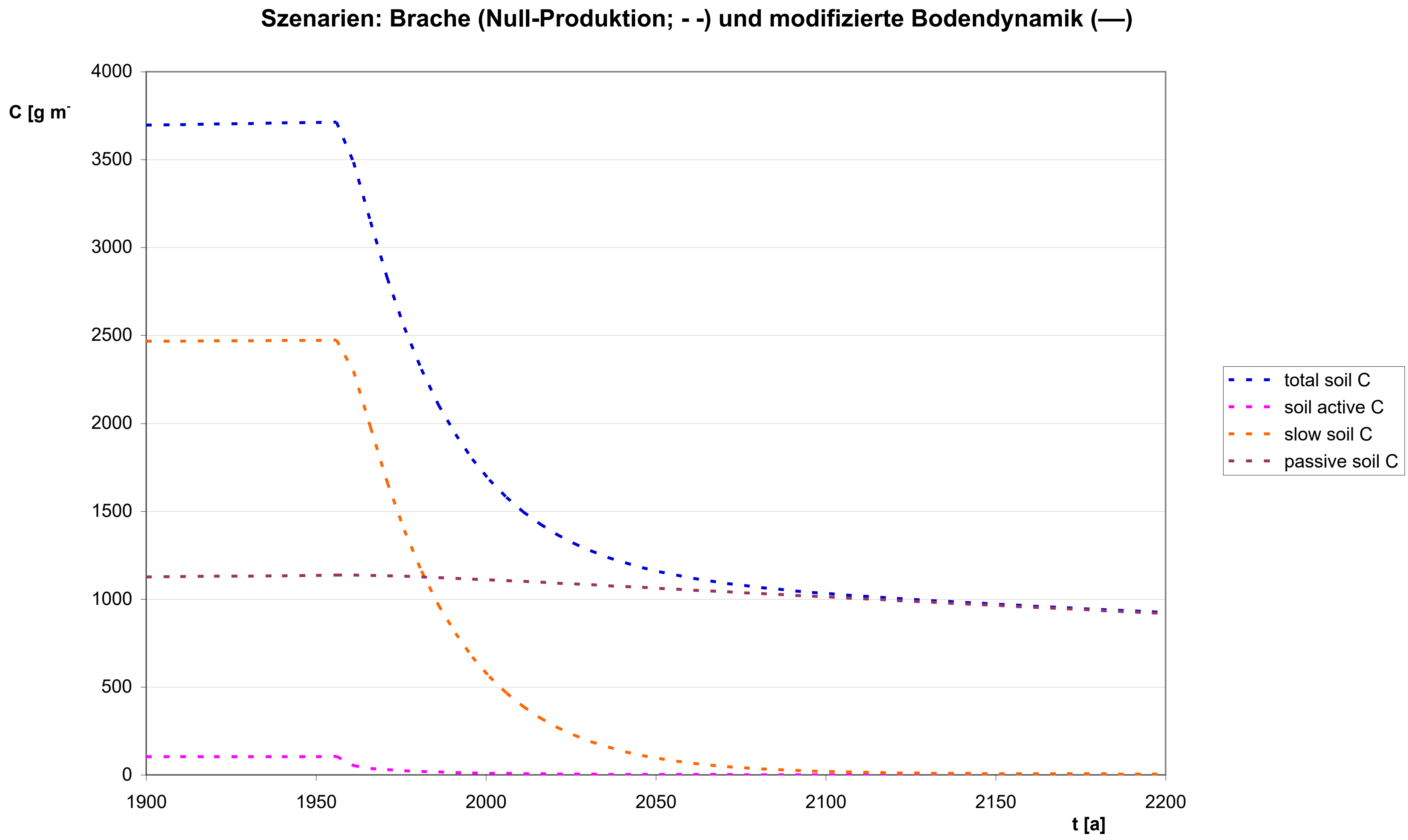


Conclusion

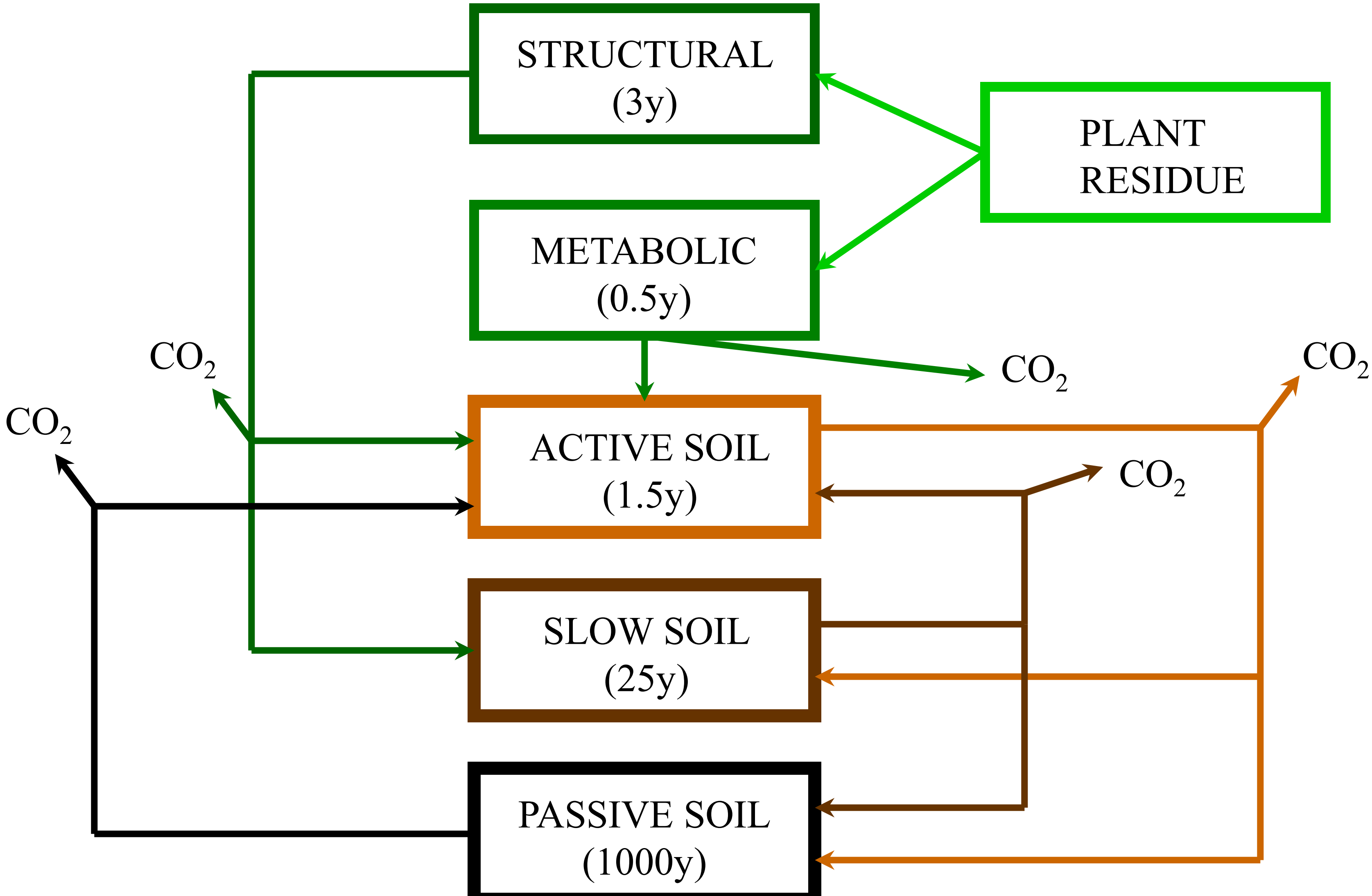
- ^{14}C age of carbon is not equivalent to molecular stability, i.e. molecules are newly synthesised using ^{14}C old carbon
- Soil microbes use all available carbon sources, however, some preferences are visible
- Some carbon source are still unknown, probably CO2 or CH4

Soil carbon models

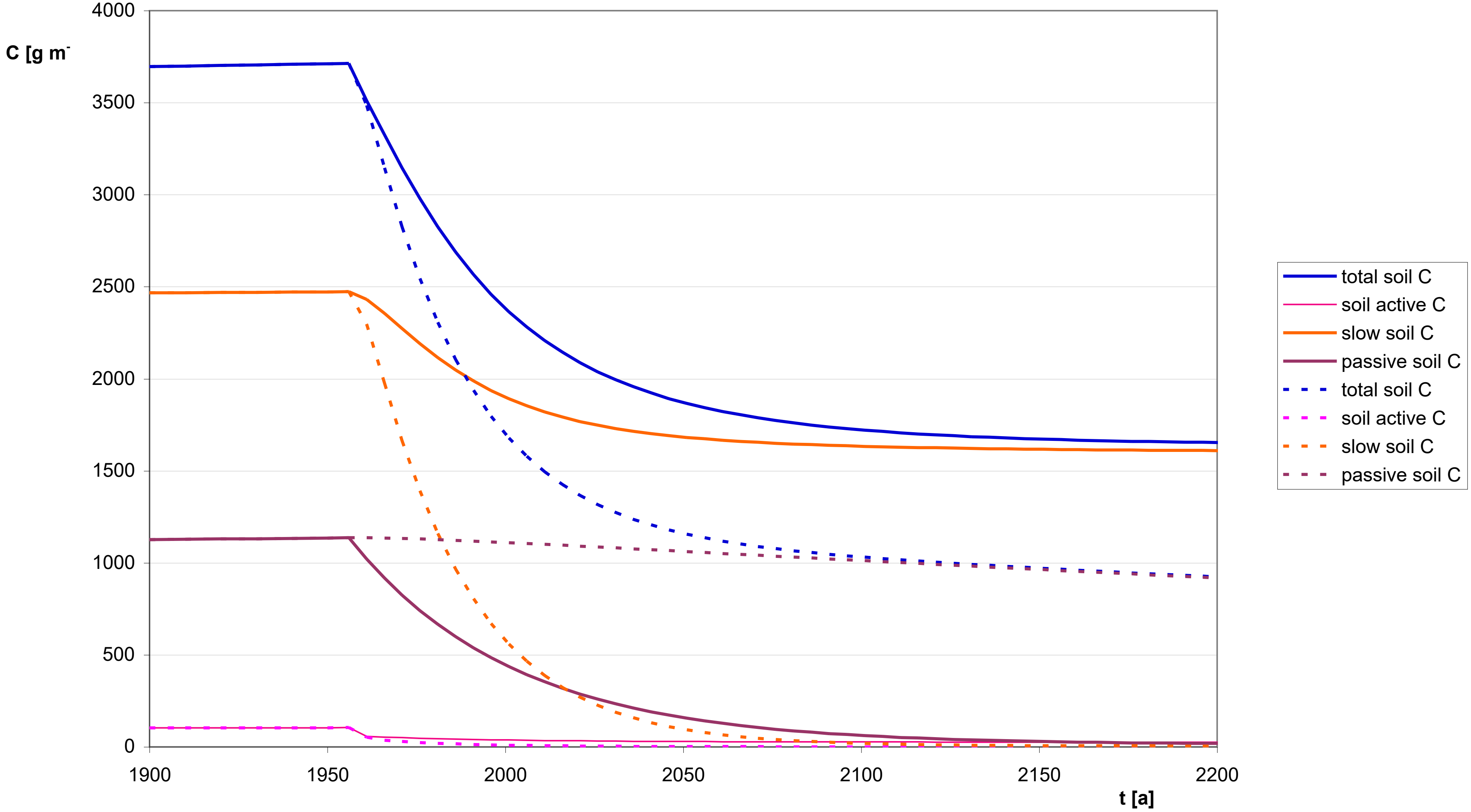


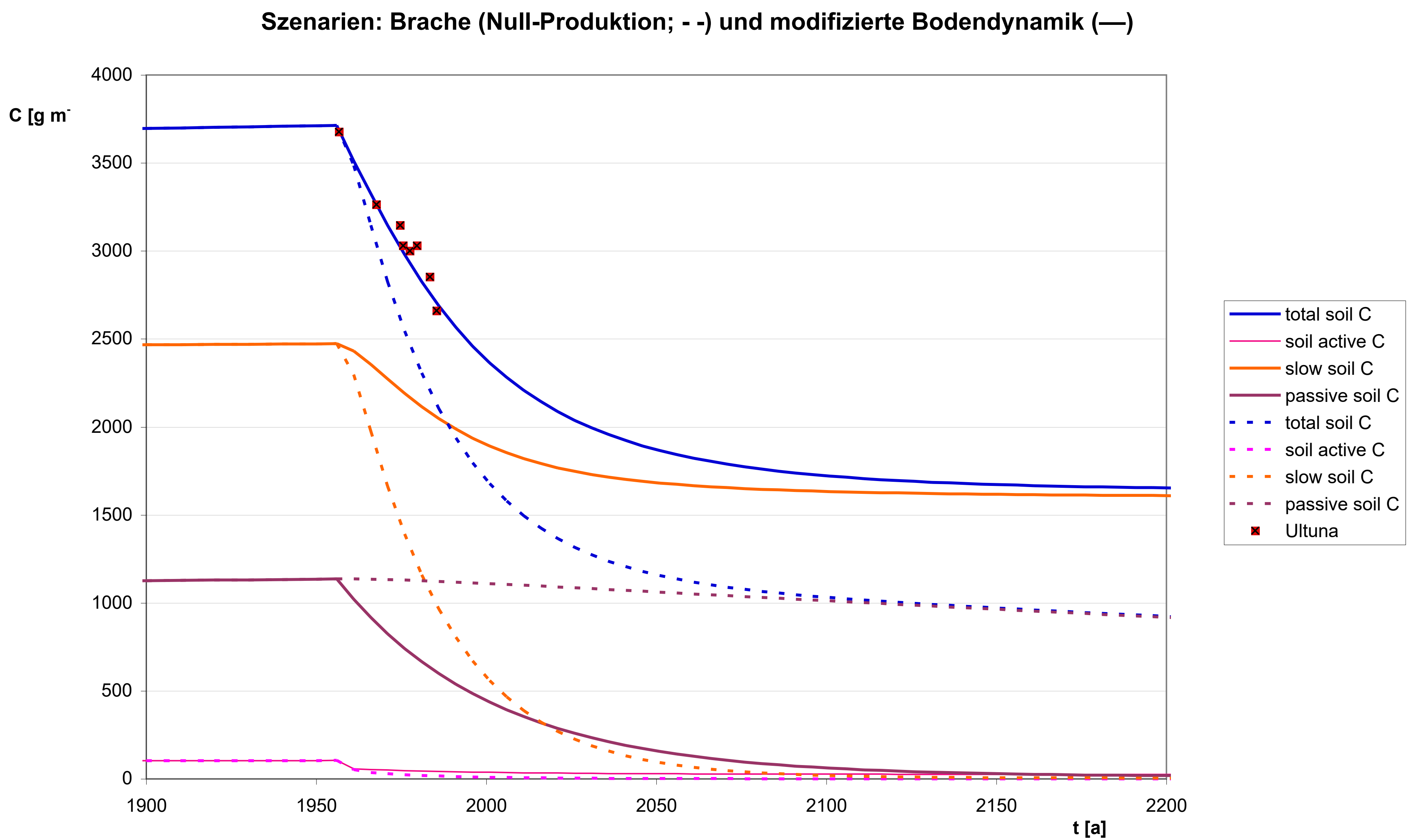

Szenarien: Brache (Null-Produktion; - -) und modifizierte Bodendynamik (—)
C [g m-
4000
3500
3000
2500
2000
1500
1000
500
0
1900
1950
2000
2050
2100
2150
2200
t [a]
total soil C
soil active C
slow soil C
passive soil C

Soil carbon models



Szenarien: Brache (Null-Produktion; - -) und modifizierte Bodendynamik (—)



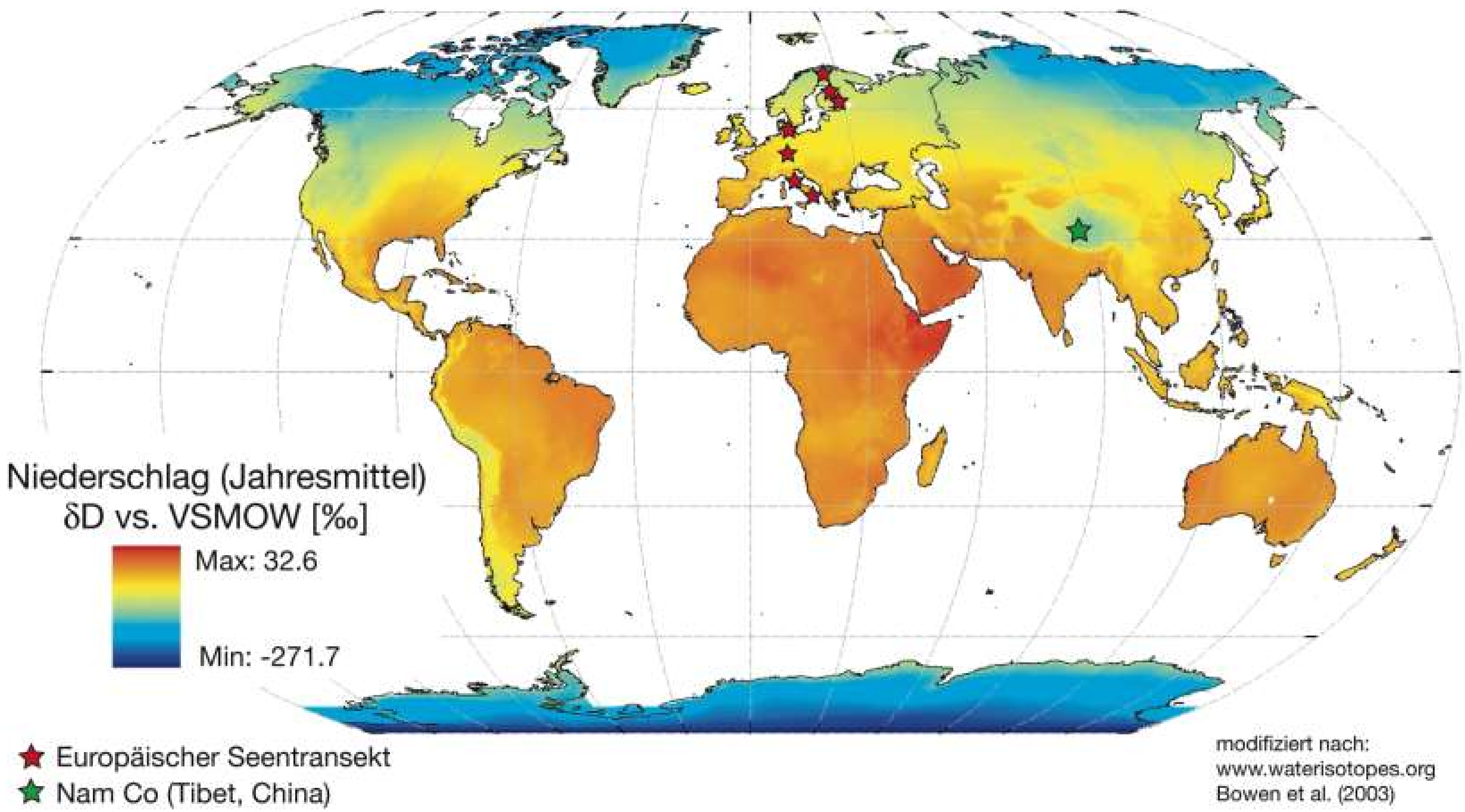


Szenarien: Brache (Null-Produktion; - -) und modifizierte Bodendynamik (—)
C [g m-
4000
3500
3000
2500
2000
1500
1000
500
0
1900
1950
2000
2050
2100
2150
2200
t [a]
total soil C
soil active C
slow soil C
passive soil C
total soil C
soil active C
slow soil C
passive soil C
Ultuna

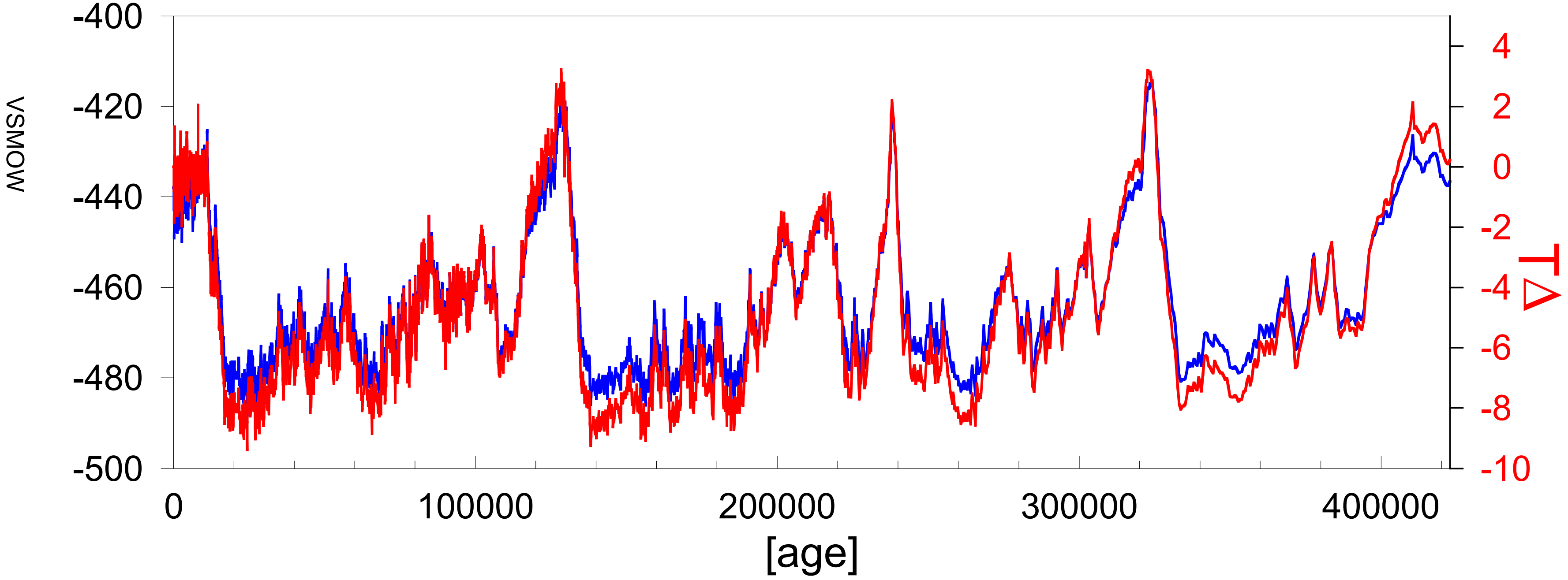
Paläoclimatology

- **Why Deuterium ?**
- Why terrestrial archives ?
- Establishing a new climatic proxy
- Case study “third pole”

Deuterium in Precipitation

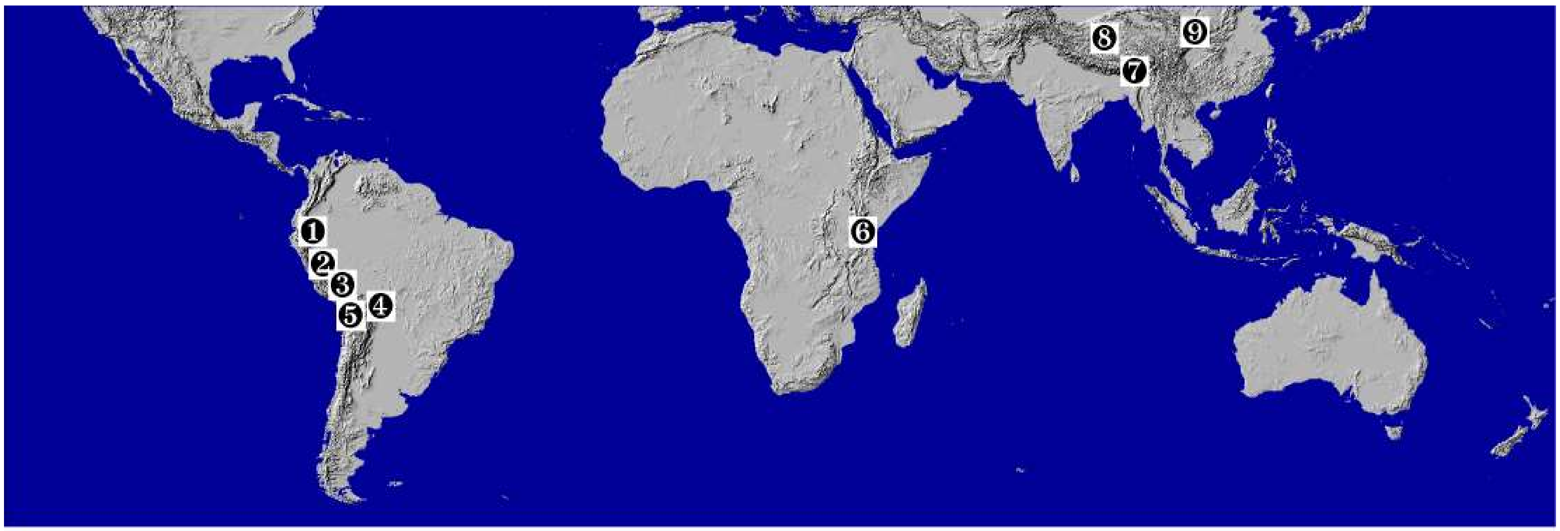


Vostok Icecore



PETIT et al. (1999) Nature, 399, 429-436.

Low Latitude Ice cores



Location of the most important stable isotope records from tropical ice cores:

❶ Chimborazo (Francou, 2000, pers. comm.) ❷ Huascarán (Thompson et al., 1995)

❸ Quelccaya (Thompson et al., 1984) ❹ Illimani (Hoffmann et al., 2002)

❺ Sajama (Thompson et al., 1998) ❻ Kilimanjaro (Thompson et al., 2002)

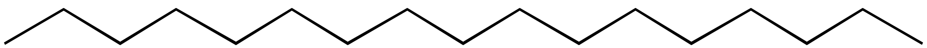
❼ Dasuopu (Thompson et al., 2000b) ❽ Guliya (Thompson et al., 1997)

❾ Dunde (Thompson et al., 1989).

(from: M. Vuille, pers. comm.)

Why trerrestrial archives ?

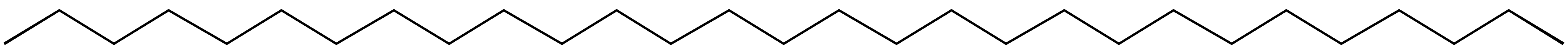
Deuterium content of Biomarkers



$C_{17}H_{36}$ (Heptadecane)

odd carbon numbered C_{17} - C_{23}: aquatic origin (algae, water plants)

$C_{23}H_{48}$ (n-Tricosane)



$C_{29}H_{60}$ (n-Nonacosane)

odd carbon numbered C_{25} - C_{31}: terrestrial origin (higher plant leaves)

Advantages of n-alkanes:

- no exchangeable hydrogen (all H is carbon bound)

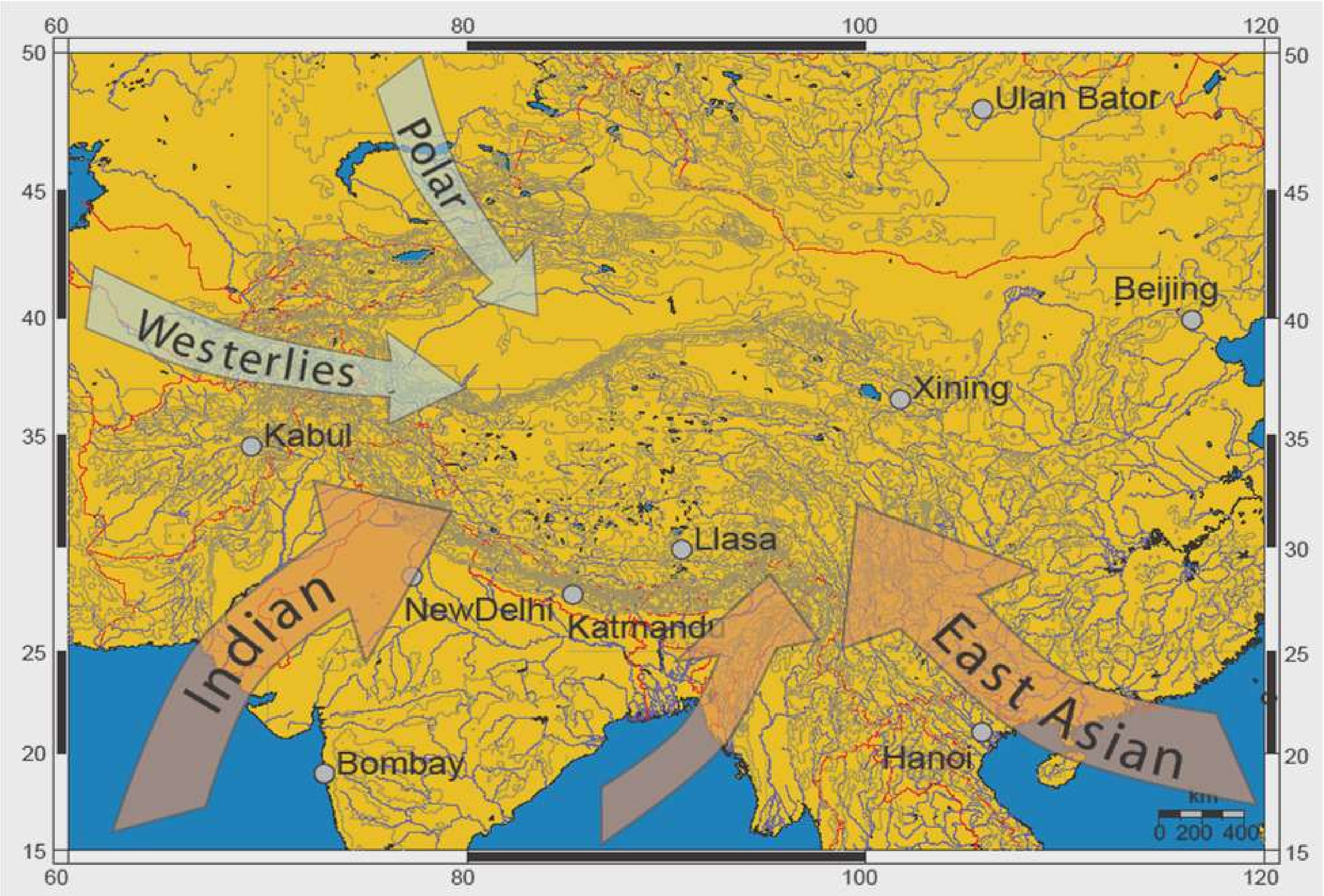
- different biological sources

- resistant, therefore abundant in sediments from the geological past

- relatively easy to extract and purify

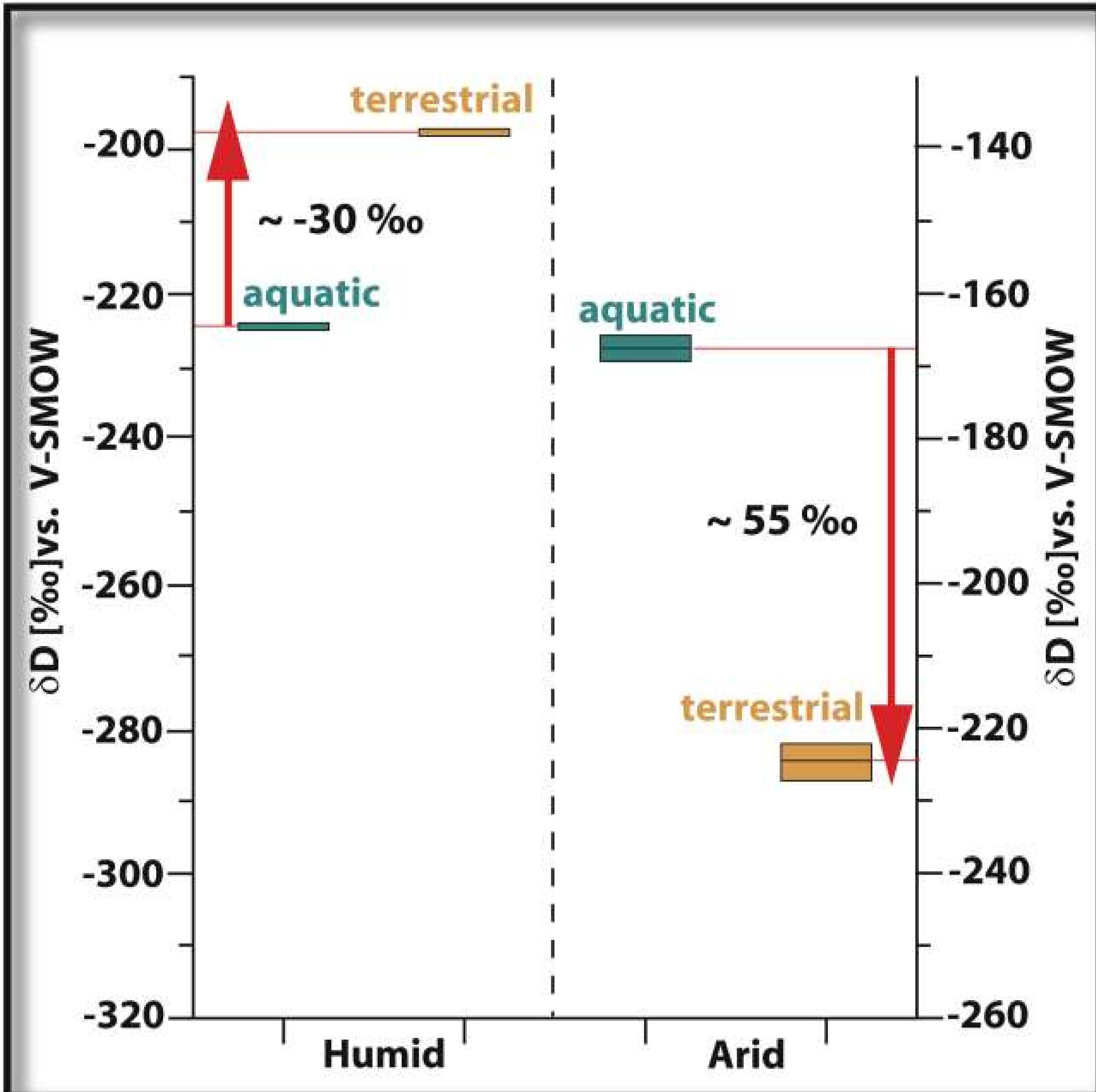
Nam Co, Tibetean Plateau

Monsoon dynamics



Overview of the study area, Lake Nam Co, Central Tibet. The esllipses mark the sediment sampling locations. (map source: Google Earth, http://earth.google.com)

Lake Nam Co, the second largest (1961 km^2) saline lake of the Tibetan Plateau is located in its central part (Fig. 2). The climate in this region is continental with low mean annual temperatures around -1° to + 3° and low precipitation amounts of 300-500 mm occurring mainly in the summer months during the monsoonal rains. Due to strong radiation annual evaporation (2465 mm) exceeds the annual precipitation.



Isotopic offset between aquatic and terrestrial n-alkanes δD values of Lake Holzmaar (humid) and Lake Nam Co (arid) sediments

Compound specific D content of biomarkers reconstructs paleohydrology


Thank you !