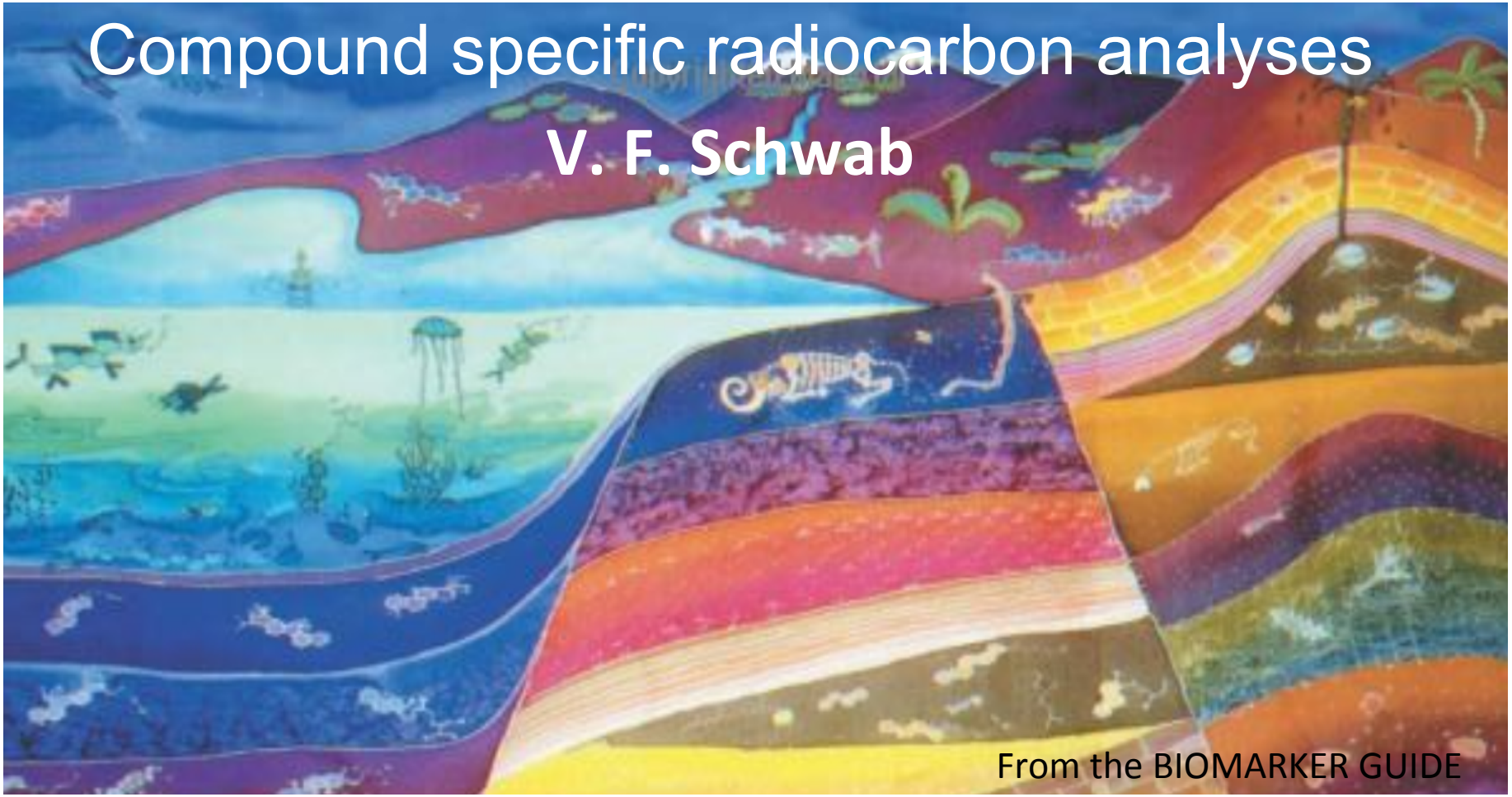
RADIOCARBON IN THE EARTH SYSTEM

Compound specific radiocarbon analyses

V. F. Schwab



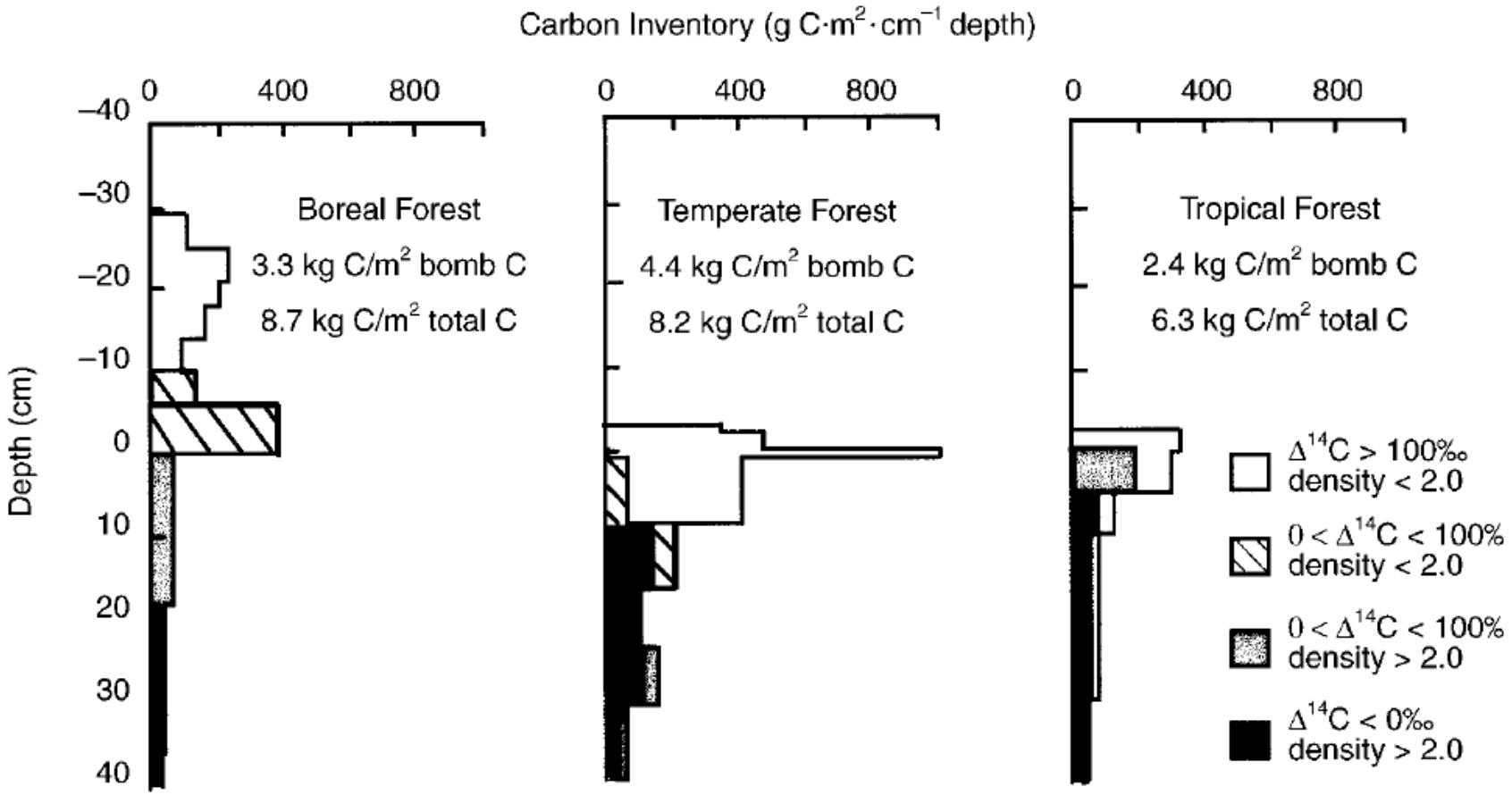
From the BIOMARKER GUIDE

MPI-BGC Jena

seit 1558

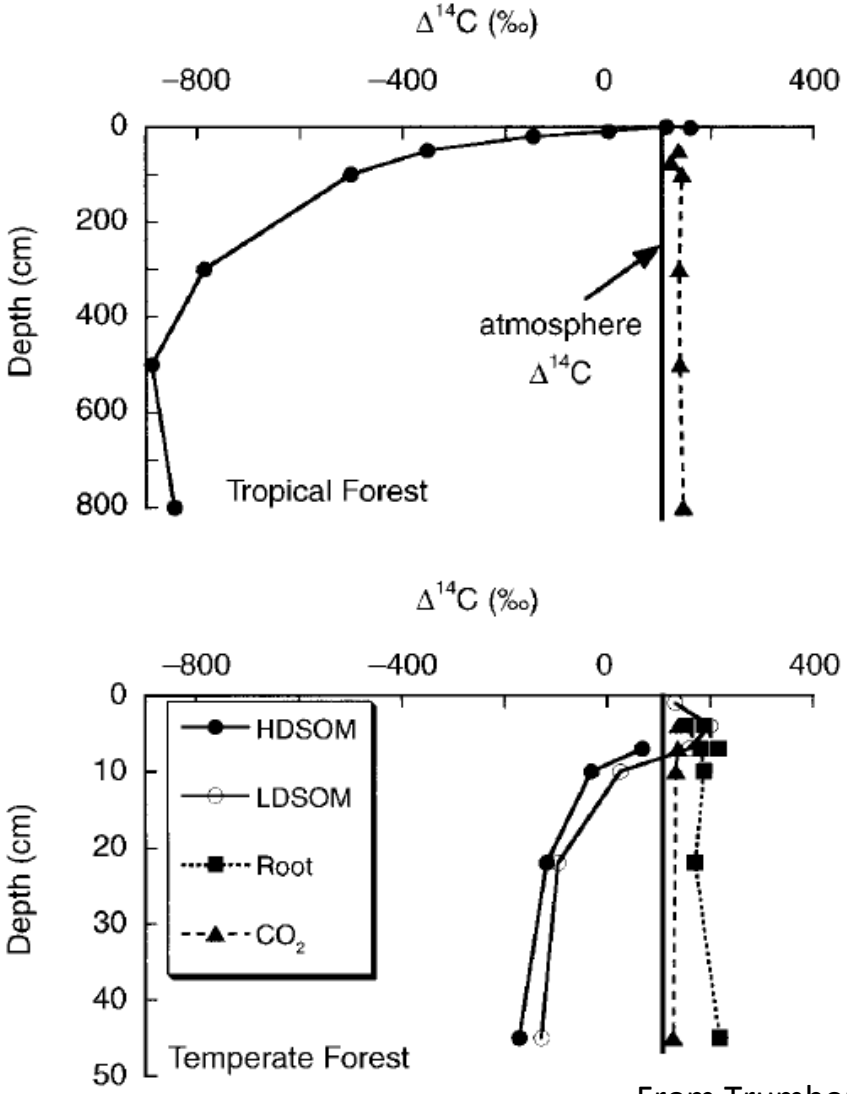
AquaDiva

Example in age variations of organic carbon pools in soils



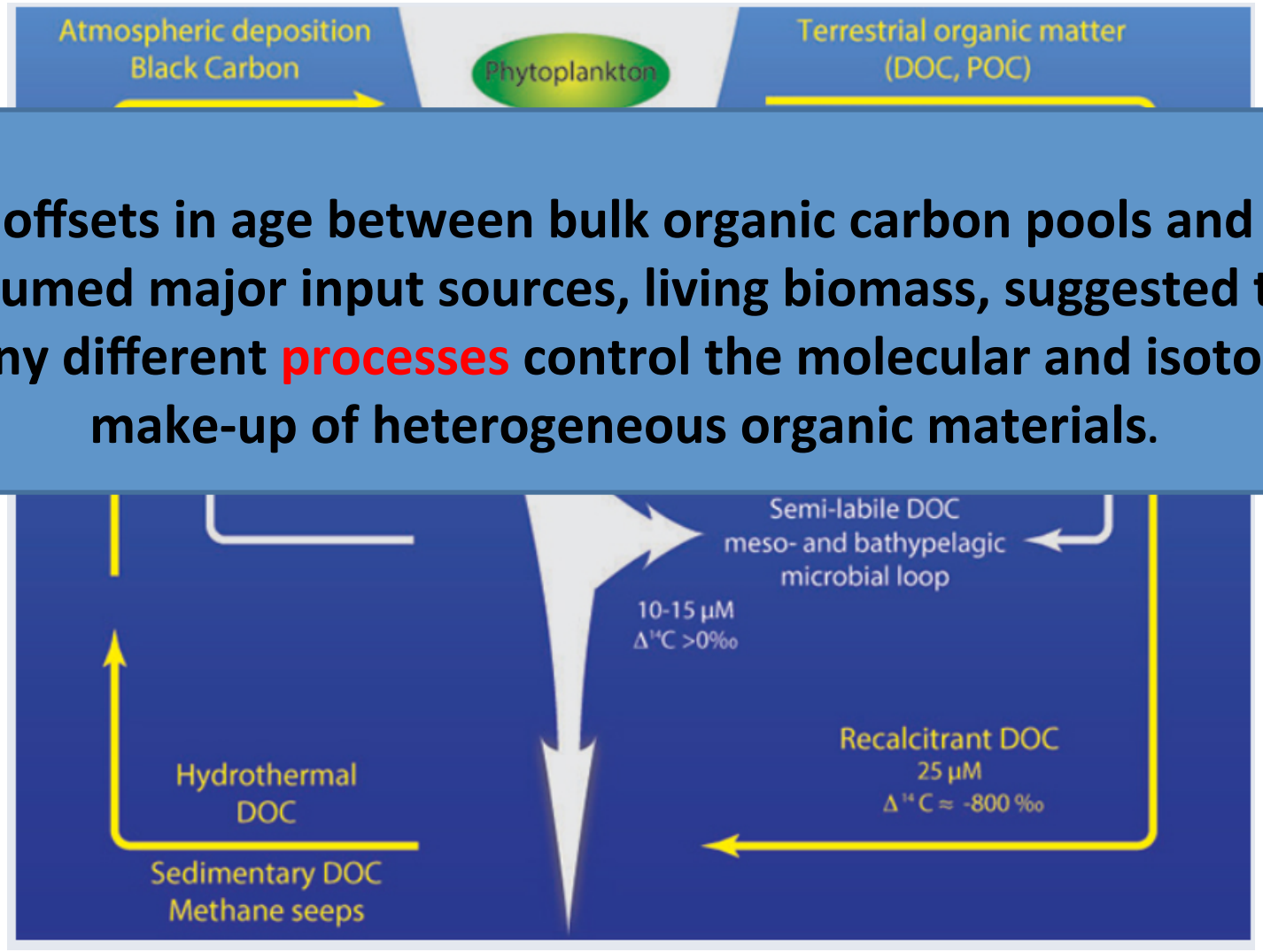
From Trumbore et al., 2000 (*Ecological Applications)*

Example in age variations of organic carbon pools in soils



From Trumbore et al., 2000 (*Ecological Applications)*

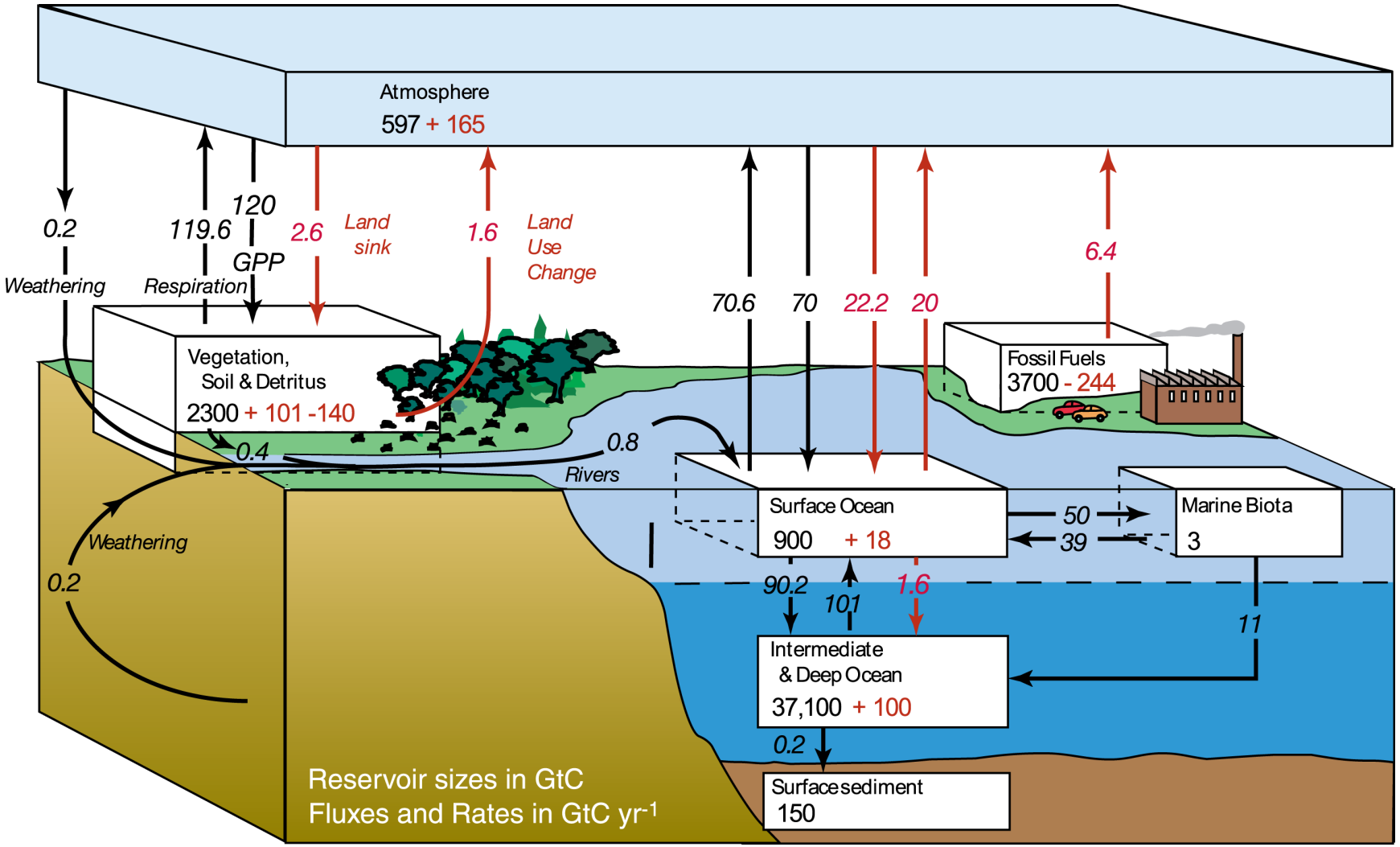
Large heterogeneity in oceanic DOC and POC



The offsets in age between bulk organic carbon pools and the presumed major input sources, living biomass, suggested that many different processes control the molecular and isotopic make-up of heterogeneous organic materials.

From http://www.whoi.edu

The Anthropogenic Carbon Cycle



https://www.gfdl.noaa.gov/anthropogenic-carbon-cycle/

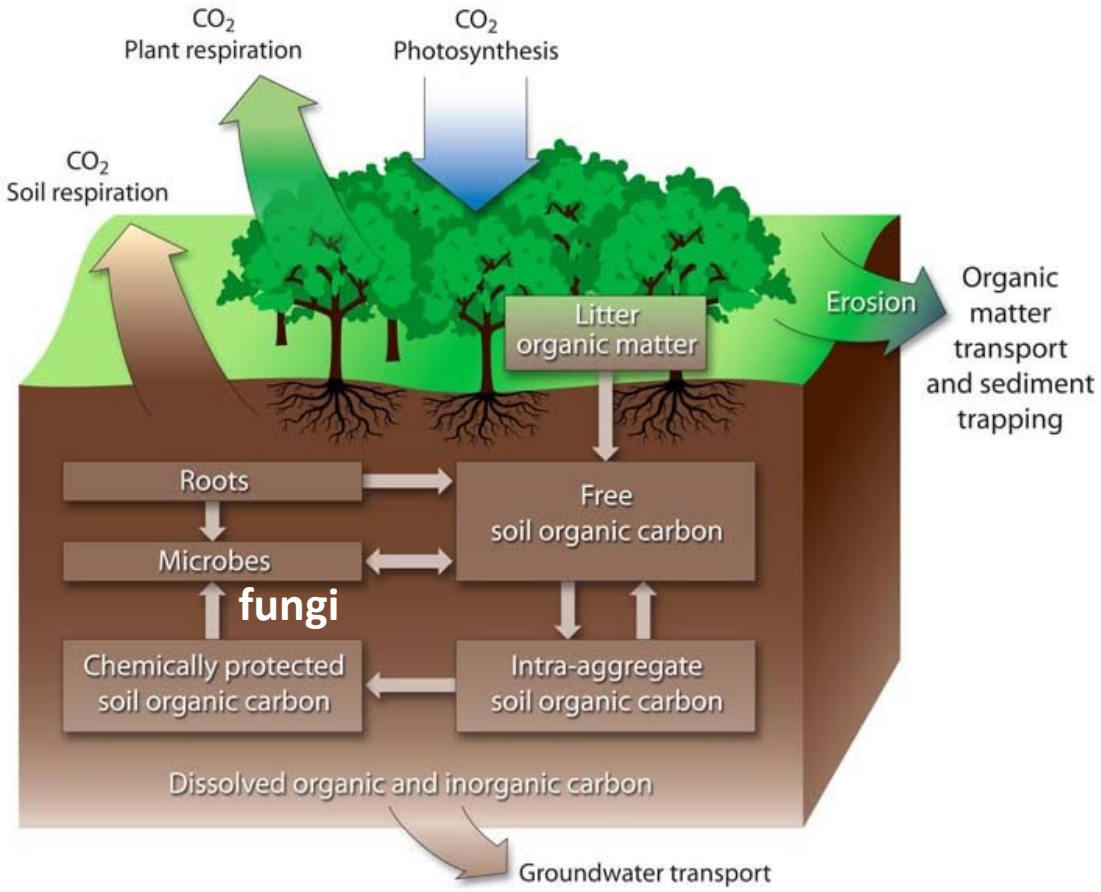
compound-specific radiocarbon analysis

Following on the heels of compound-specific stable isotope analysis (GC-C-IRMS) compound-specific radiocarbon analysis (CSRA) is envisioned as a way to couple the diversity of carbon sources with the residence time of carbon in the respective source pools. (Ingalls and Pearson. Oceanography (2005))

Applications of CSRA include studies of the

- fixation, transformation, transport, and preservation of organic carbon
- elucidation of microbial metabolic pathways
- sources and reactivity of dissolved organic carbon
- organic paleo-proxy dating
- development of improved sediment chronologies.

Single-compound radiocarbon analysis help to couple the diversity of carbon sources with the residence time of carbon in the respective source pools.



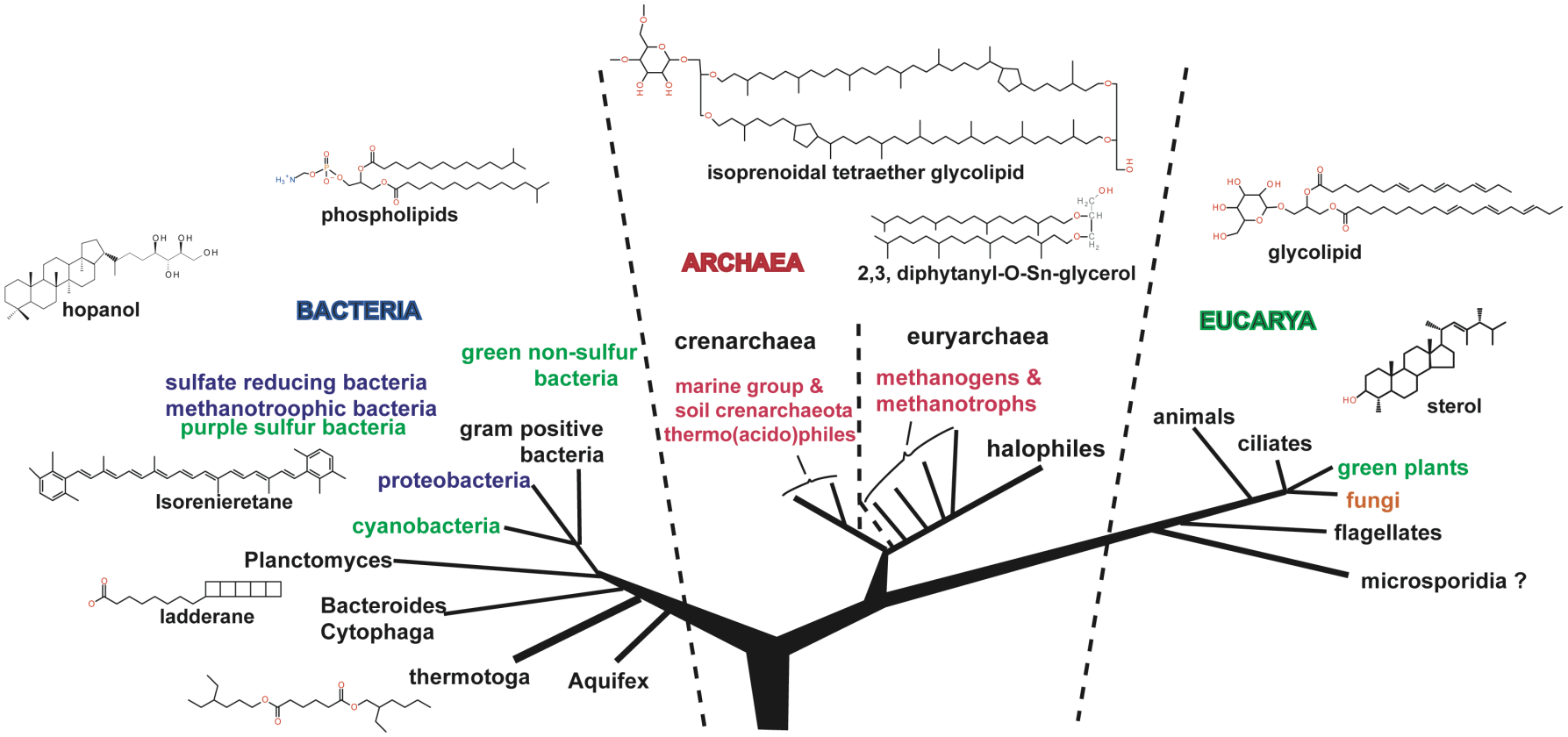
CSRA MAY HELP TO IMPROVE MODELs

What is a biomarker in environmental and climate science

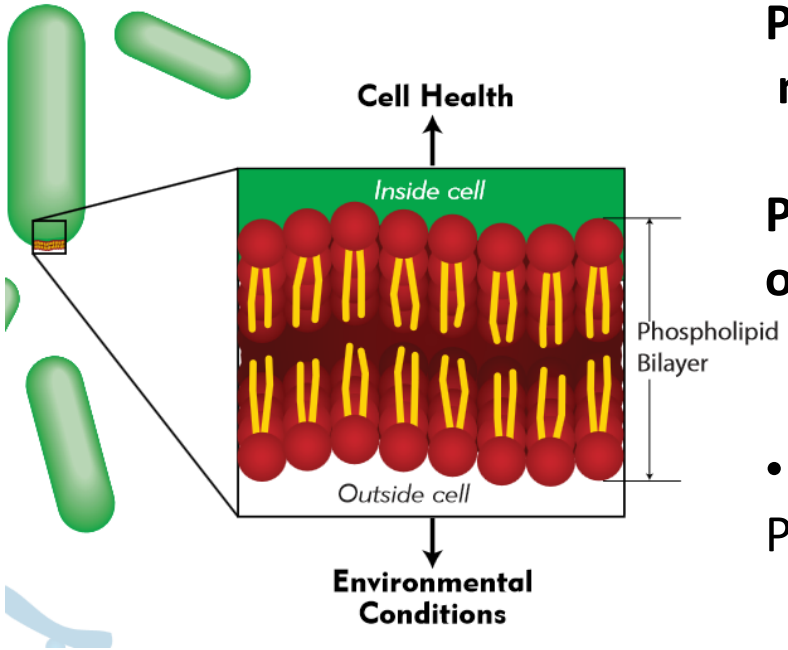
Molecular biological markers, or **biomarkers**, are natural products that can be traced to a particular biological origin.

Organic compounds with **specific biological sources**, whose structures can be **preserved** through geologic time.

Example of BIOMARKERS



What are PLFAs



Phospholipid fatty acids (PLFAs) are a main component of cell bacteria.

PLFAs analysis provides direct information on the entire microbial community.

- Biomass

PLFAs represent all living cells.

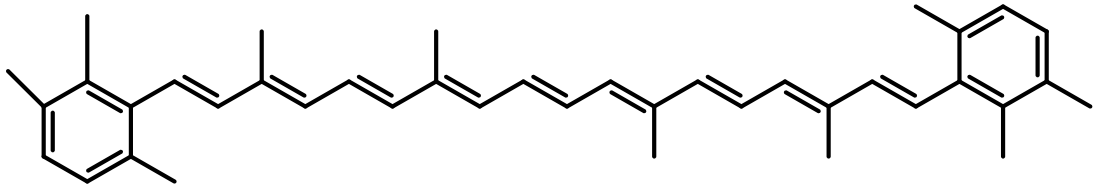
- Population "Fingerprint"

Some organisms produce specific or signature types of PLFA biomarkers allowing quantification of important microbial functional groups (e.g. iron reducers, sulfate reducers, or fermenters).

PLFAs: as microbial population fingerprint

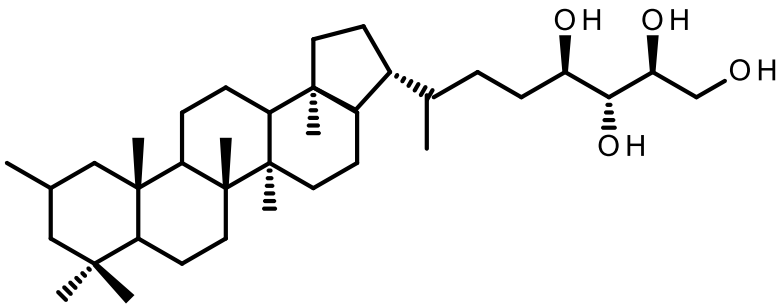
PLFAs Type	Bacterial group	Potential relevance
Monoenoic (Monos)	Gram-negative; Proteobacteria (aerobes and anaerobes)	e.g. Hydrocarbon utilizing or Nitrogen fixing bacteria
Terminally branched Saturated	Firmicutes and Bacteroides	Firmicutes: anaerobic fermenting bacteria
Branched monoenoic	in sulfate reducing bacteria and Planctomycetes	Desulfobacter
Mid-Chain Branched Saturated	In sulfate reducing bacteria and *Actinomycetes*	Often associated with iron reducing bacteria
Polyenoic (Polys)	Found in eukaryotes Eukaryotic (fungi, algae, protozoa, plants and animals)	Eukaryotic scavengers often prey on contaminant utilizing bacteria

Some more specific bacteria lipids



isorenieratene

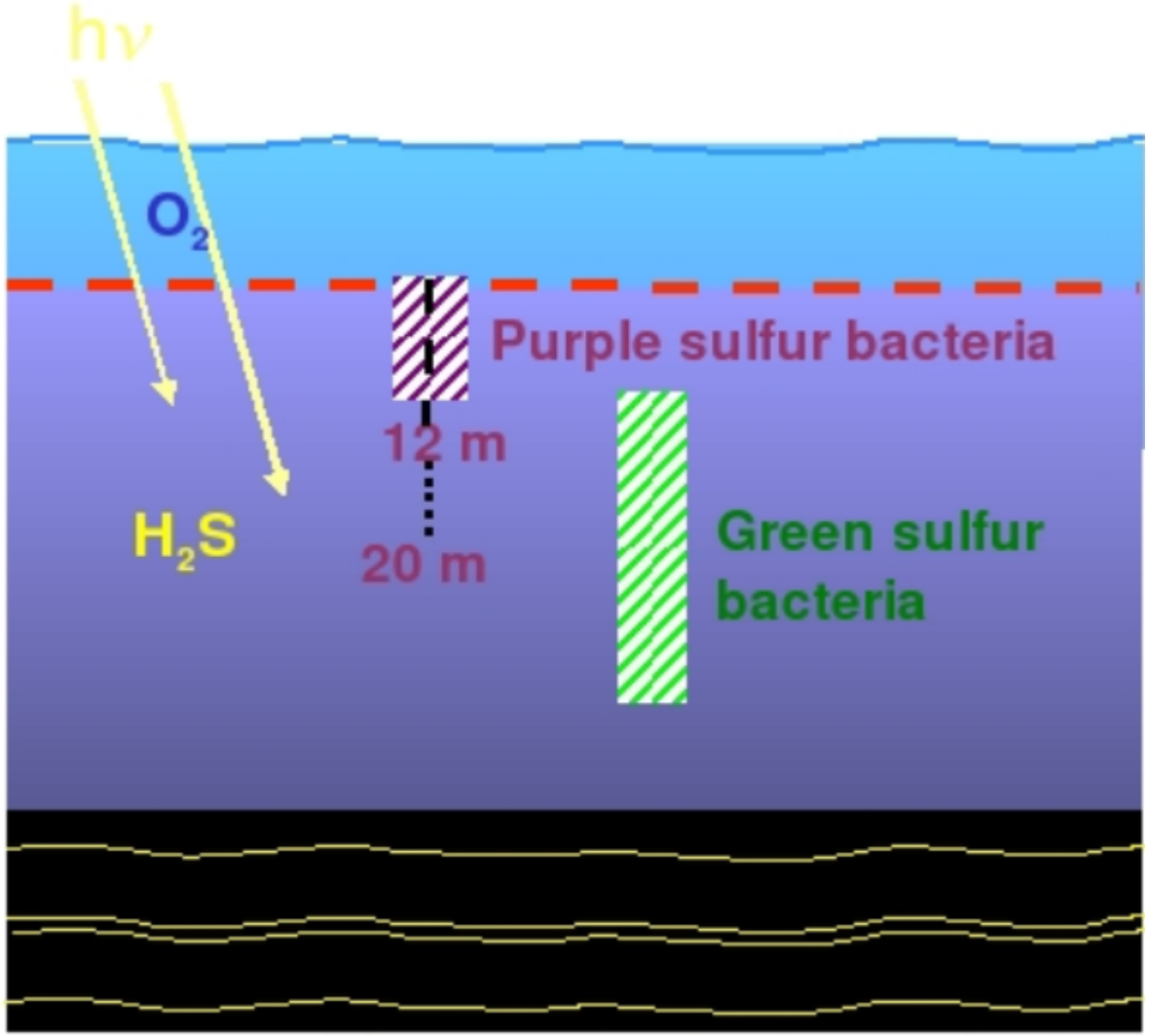
Green purple sulfur bacteria

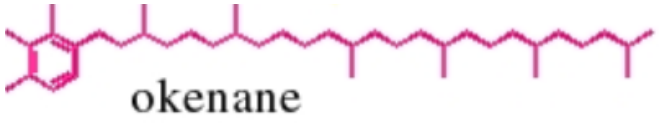


2-methyl hopanol

cyanobacteria

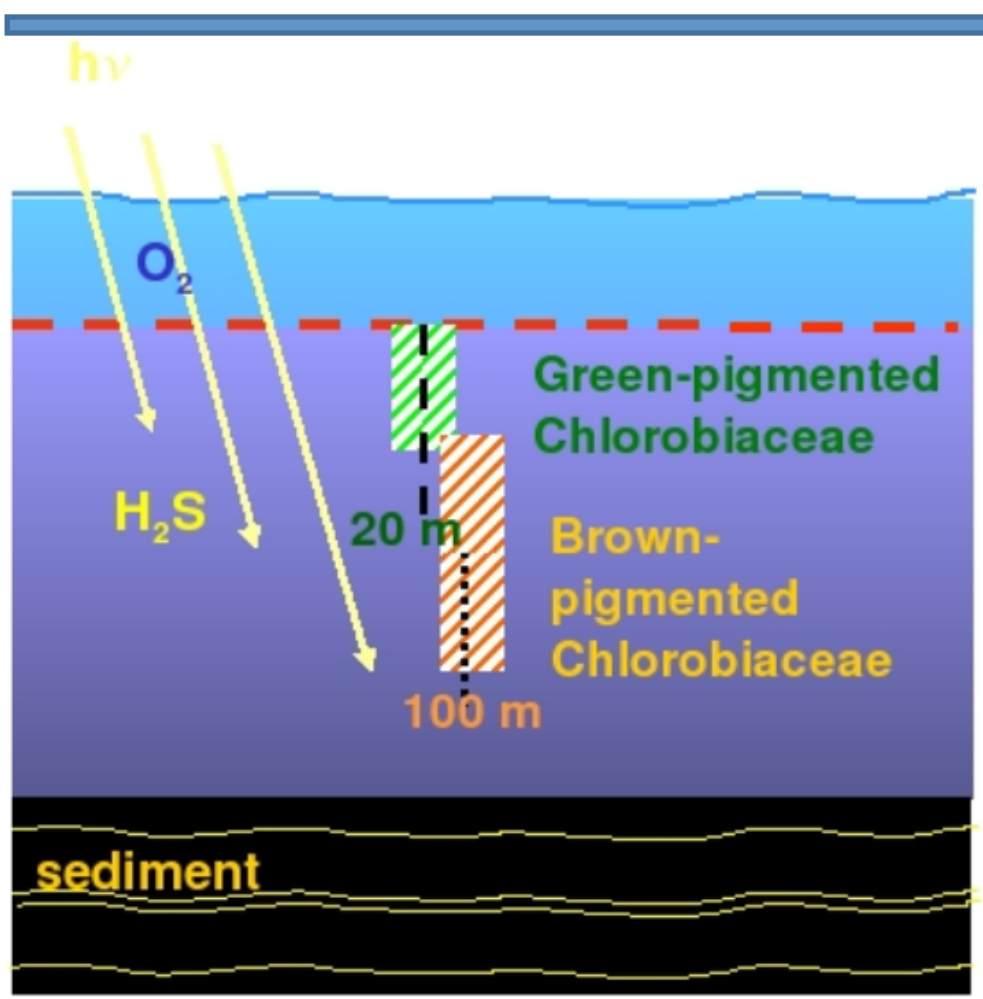
Some more specific bacteria lipids

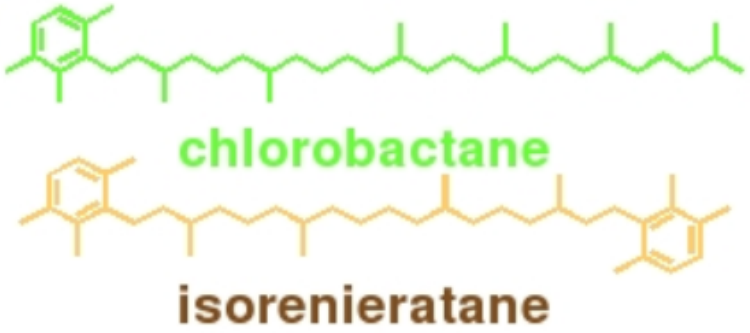




Summons et al., 1987

Some more specific “bacteria” lipids

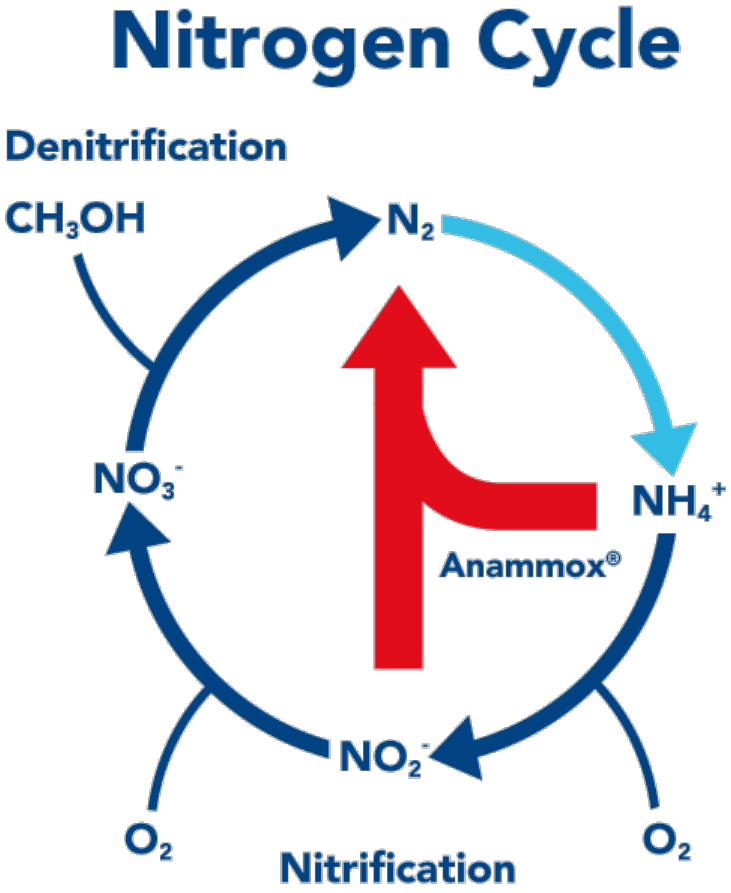




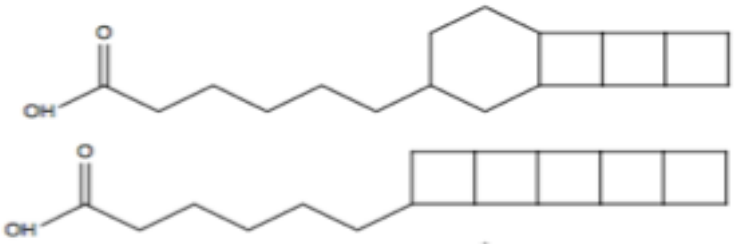
Summons et al., 1987

Some more specific bacteria lipids

Ladderanes (PLFAs) derived from anammox bacteria,
an abbreviation for **AN**aerobic **AMM**onium **OX**idation

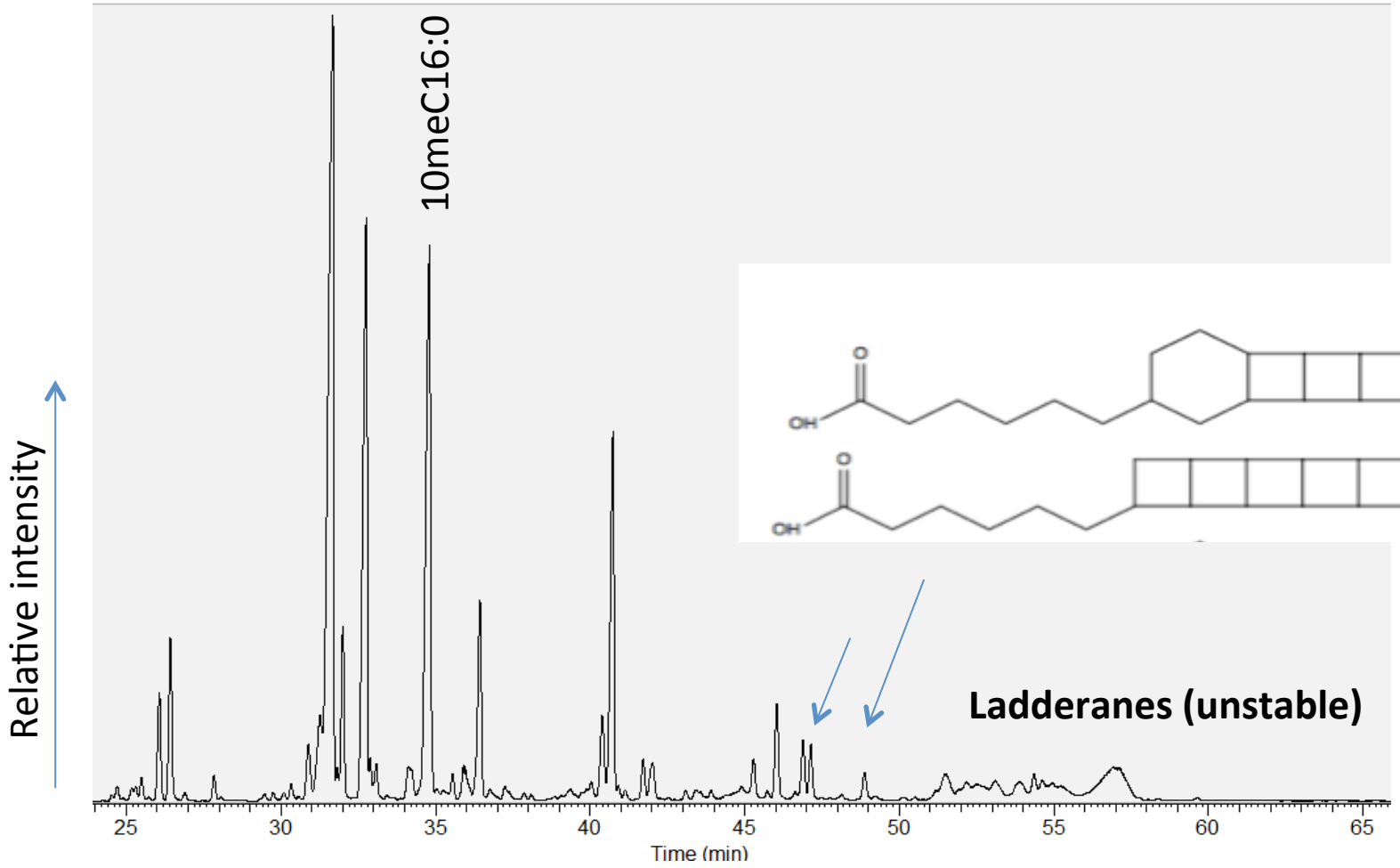


Ladderanes

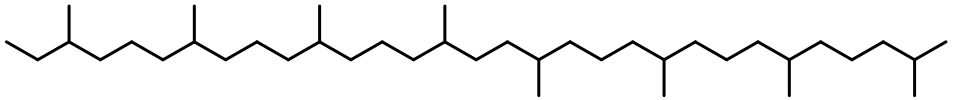


They have been proposed to be responsible of up to 70% of oceanic N_2 production, representing a major N sink.

Example of PLFAs profile (groundwater)

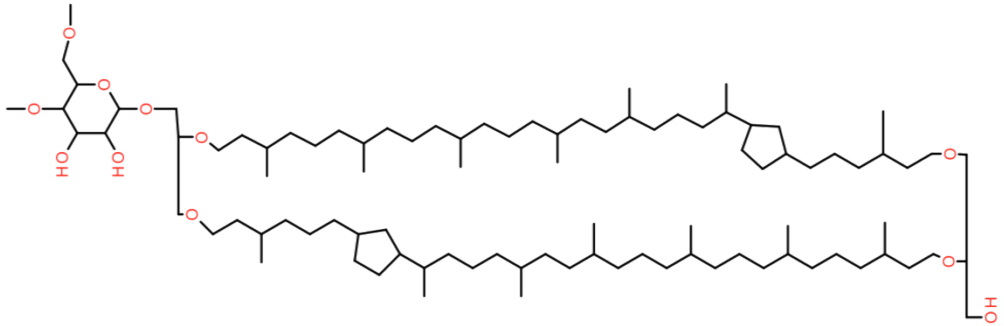


Some more specific archaea lipids



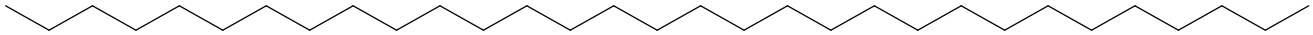
byphytane

Methanotrophic archaea



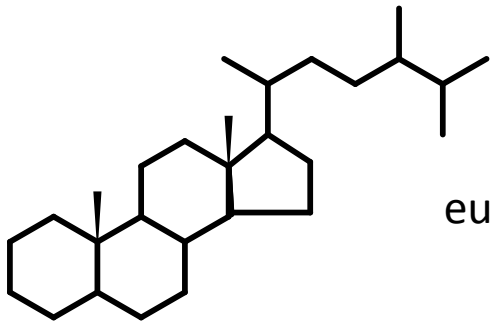
Glycerol dibiphytanyl glycerol *tetraether* (*GDGT)*

More specific prokaryote lipids

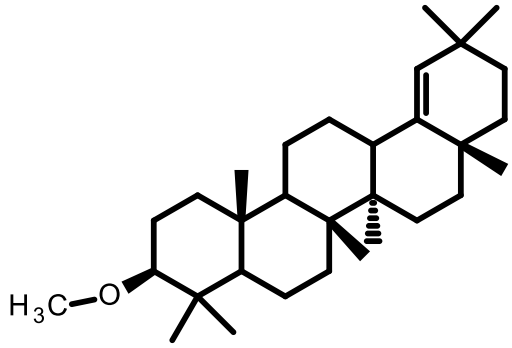


Long chain *n*-alkanes

Terrestrial plants

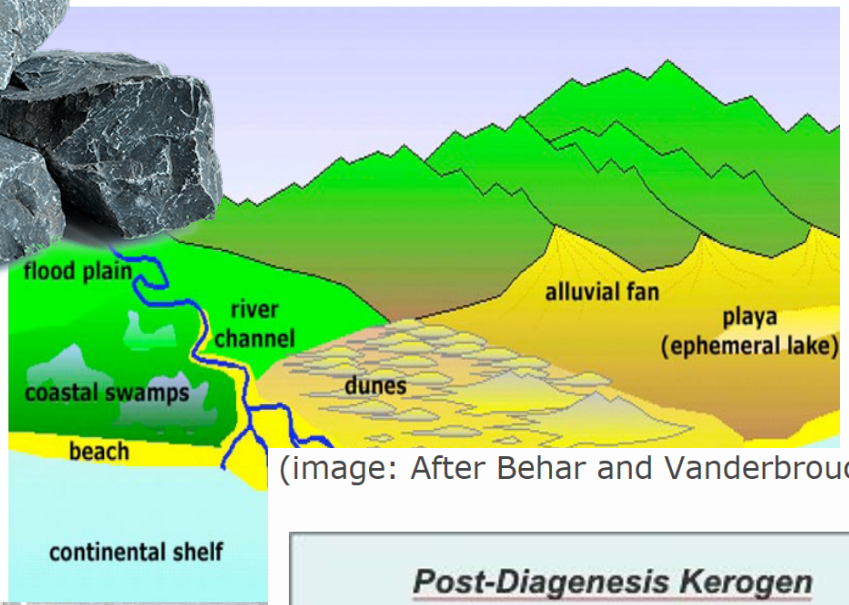


eucaryotes

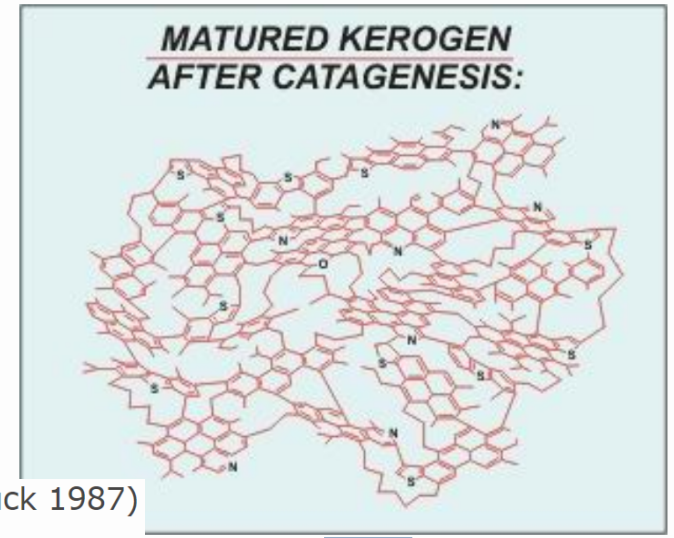


miliacine

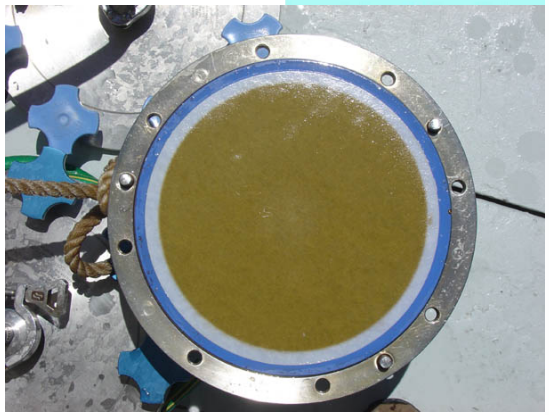
Formation and origin of biomarkers

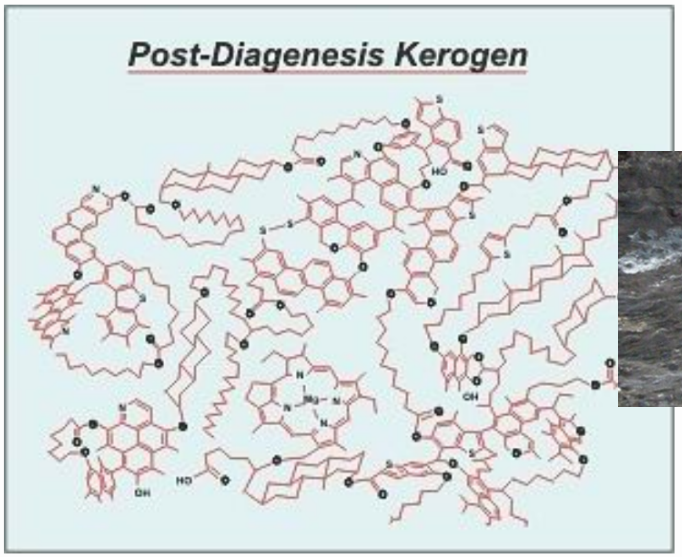


(image: After Behar and Vanderbrouck 1987)



(image: After Behar and Vanderbrouck 1987)





Free lipids

General workflow

- Biological
- Environmental
- ---

- Liquid extraction
- Solid phase extraction
- Filtration
- Purification

- CE (capillary electrophoresis)
- LC (liquid chromatography)
- GC (Gas chromatography)

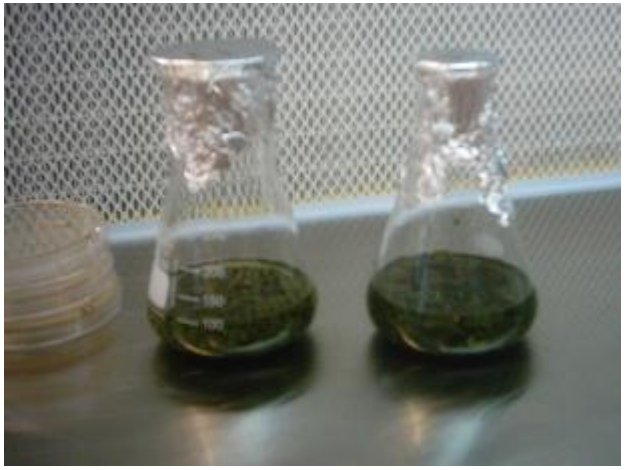
- Global profiling
- Targeted approaches

- Identification
- Quantification

- Detection of significant influences

Lipid extract

Mature samples



Pant extract

Typical purification of mature samples

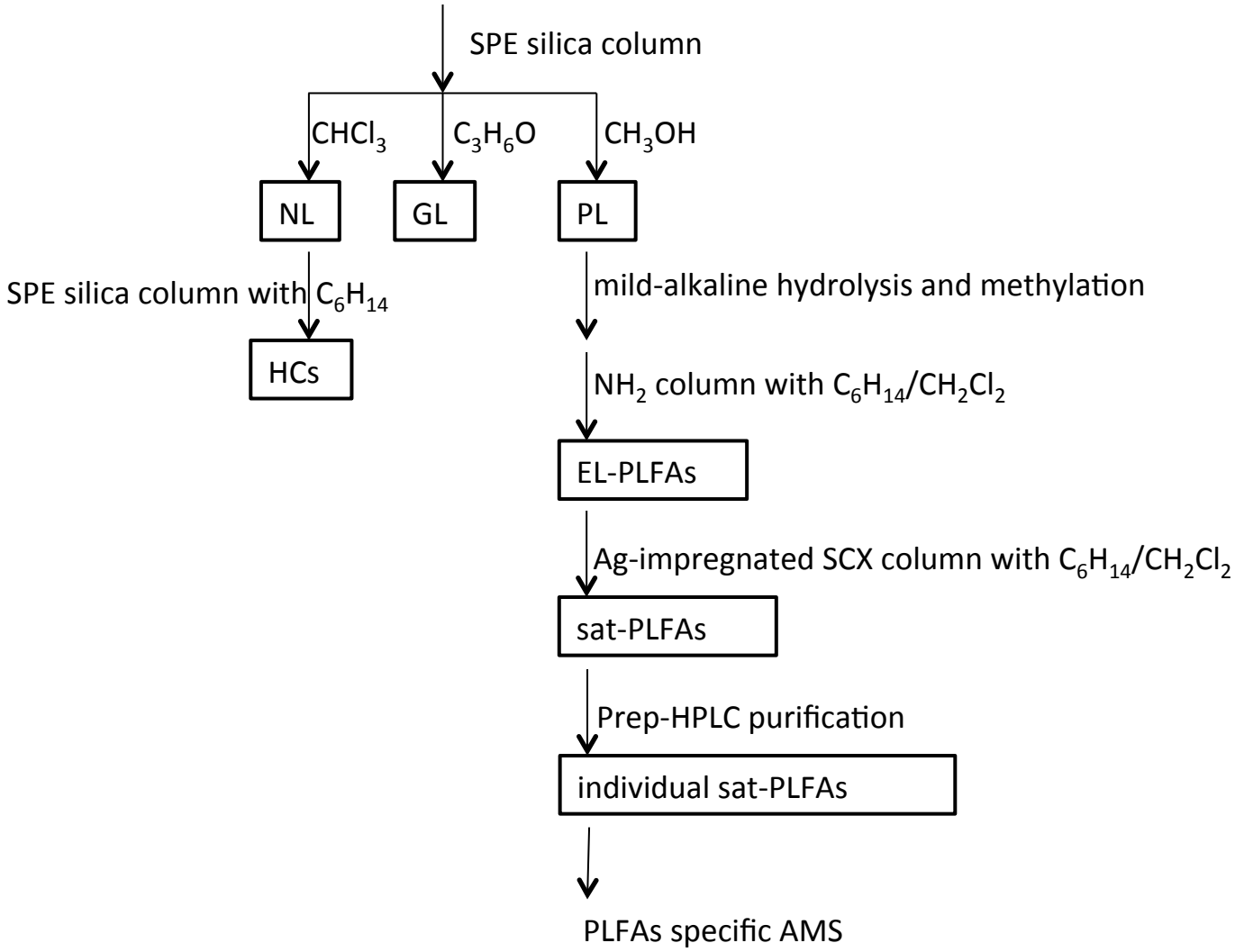
Extraction (DCM/MeOH 9/1)

Total lipids extract (TLE)

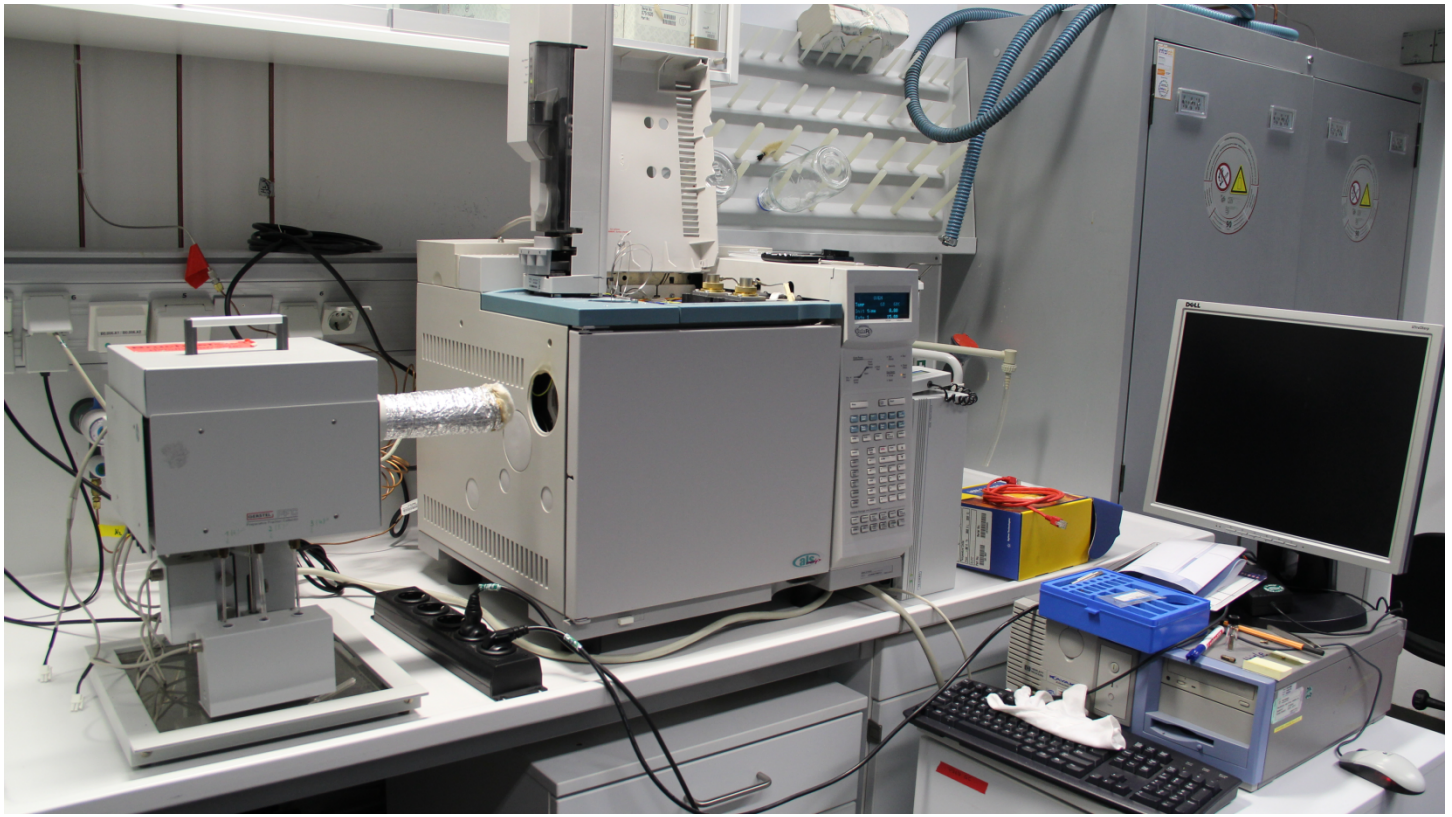
Liquid chromatography (activated silica or alumina)

Hexane/DCM (9/1)	Hexane/DCM (1/1)	DCM/MeOH (1/1)
F1 Saturated hydrocarbon (*n*-alkanes)	F2 ester/ketone miliacin alkenones	F3 Alcohol disnosterol hopanols
GC-MS	GC-MS	GC-MS

Typical purification of fresh samples



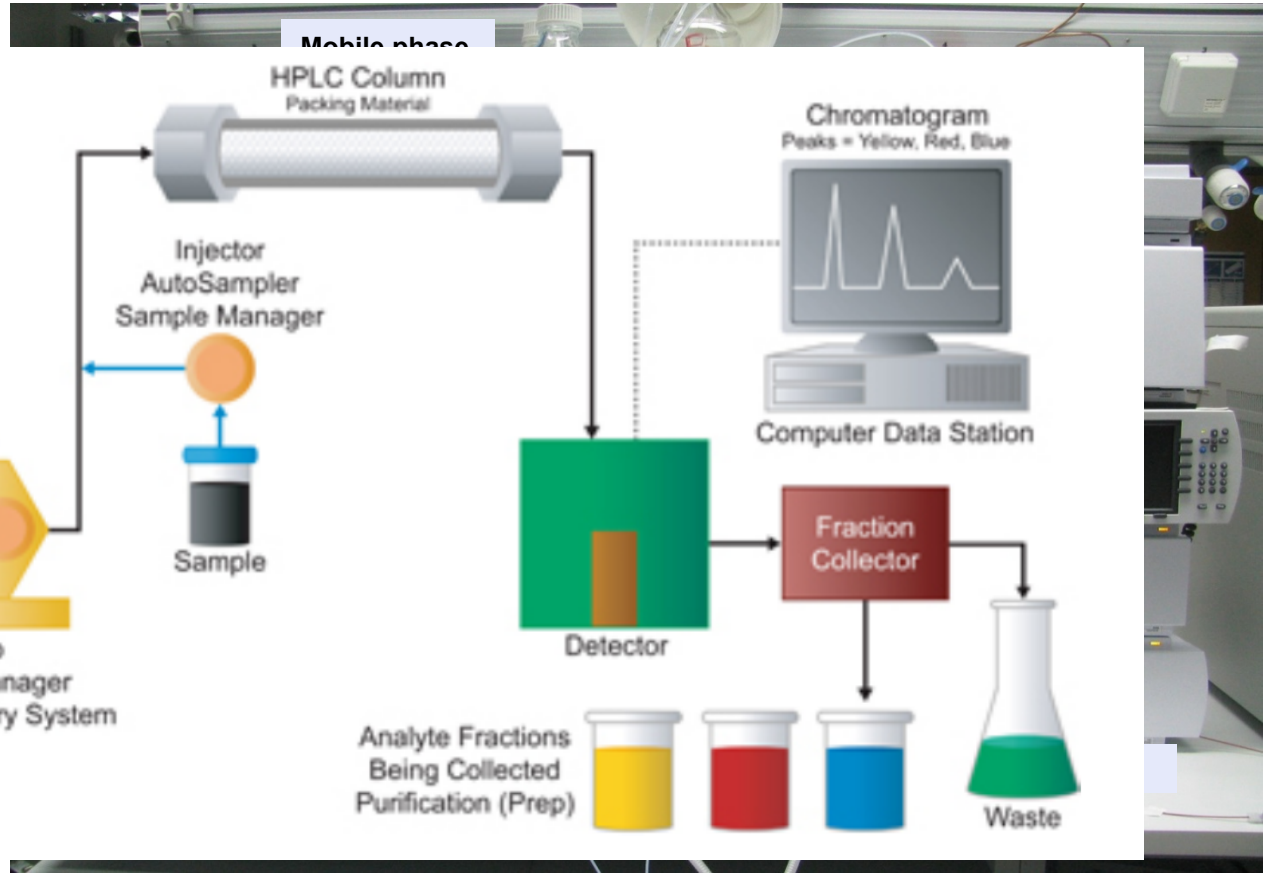
Compound purification –prep –GC



Compound purification –prep – HPLC

HPLC-MS (quadrupole)

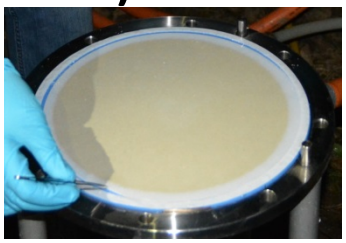
MS (ESI)



HPLC

Chemical treatment

Samples are frozen (-80°C) until analyze



Lipid extraction

Chemical pre-purification

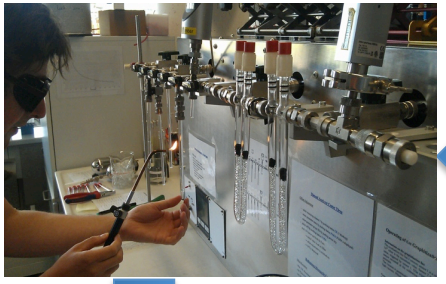
GC-TQMS

Lipids identification

Prep-HPLC

Lipids isolation

Extraction lines



AMS

$\Delta^{14}C$ PLFAs

Comparison between HPLC and GC

Sample volatility

HPLC
No volatility requirement

Sample must be soluble in mobile phase

GC
Sample must be volatile

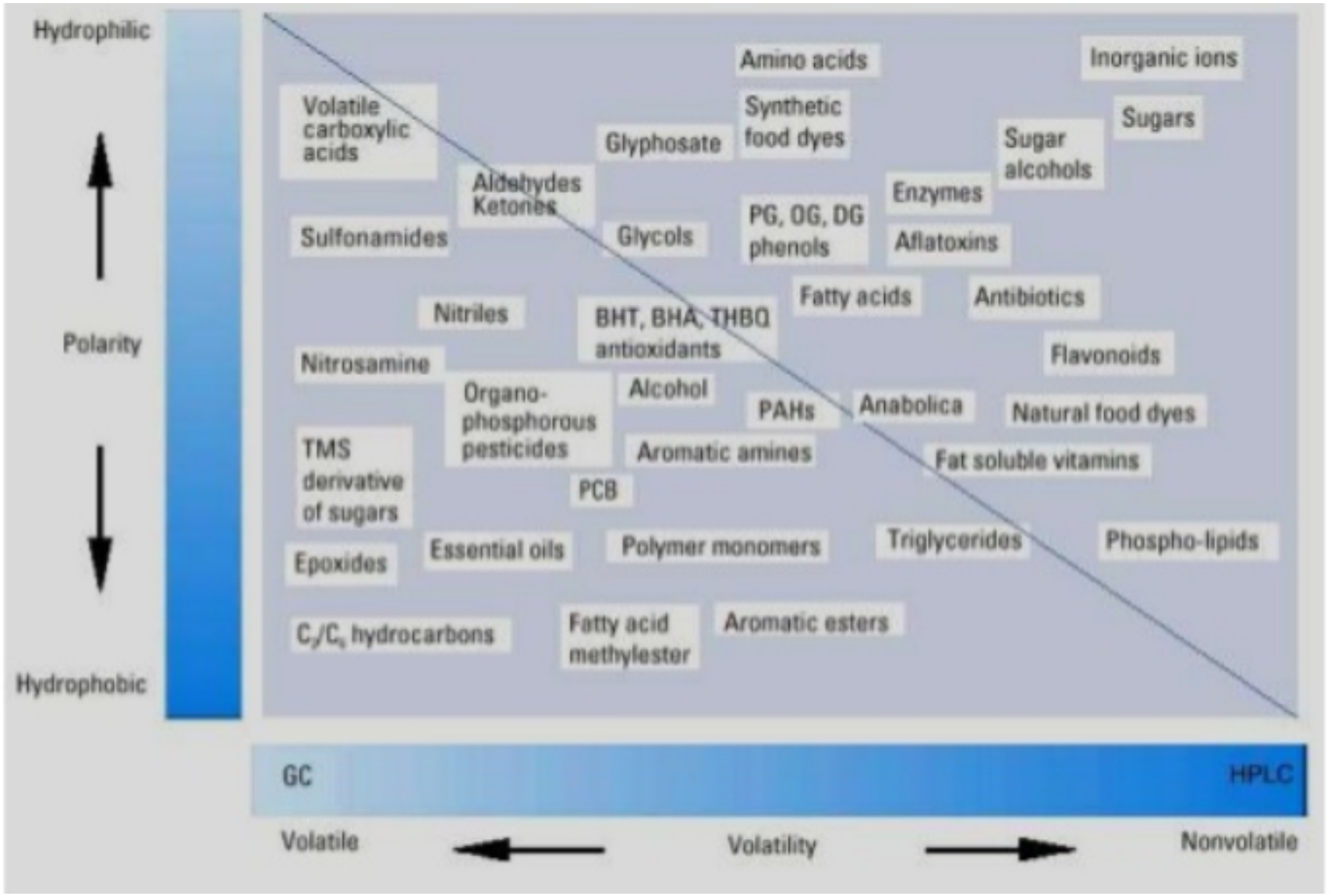
Sample Polarity

HPLC
Separates both polar and non polar compounds

PAH-inorganic ions

GC
Separates both polar and non polar compounds

Comparison between HPLC and GC



Comparison between HPLC and GC

Sample thermal lability

HPLC
Close to room t emperature

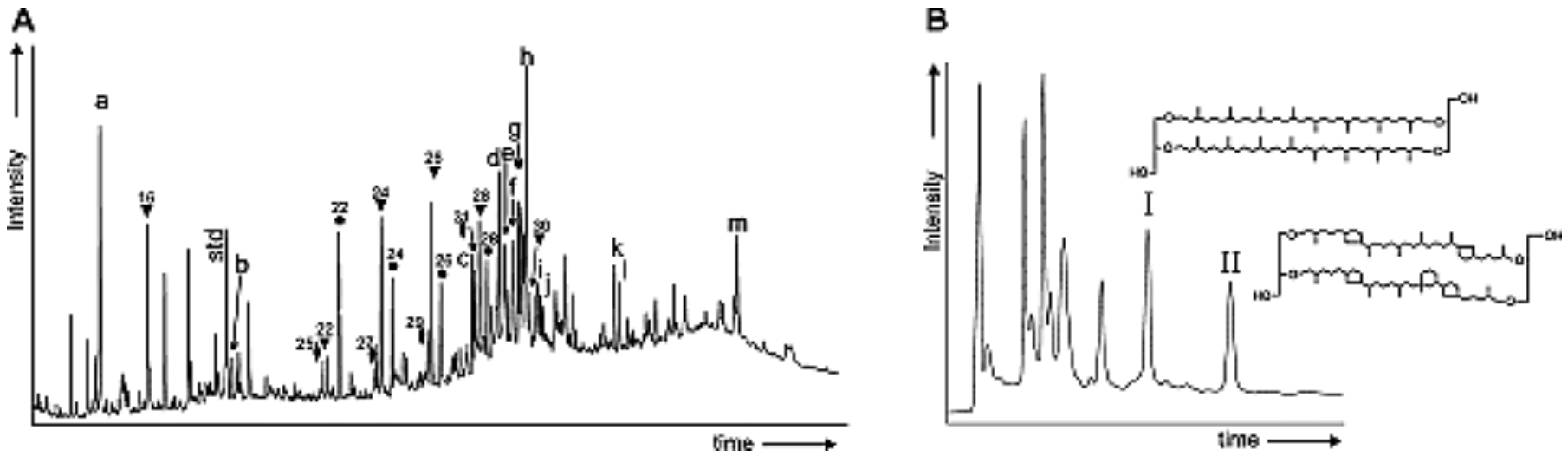
GC
Sample must be able to survive high temp. injection

Sample molecular weight

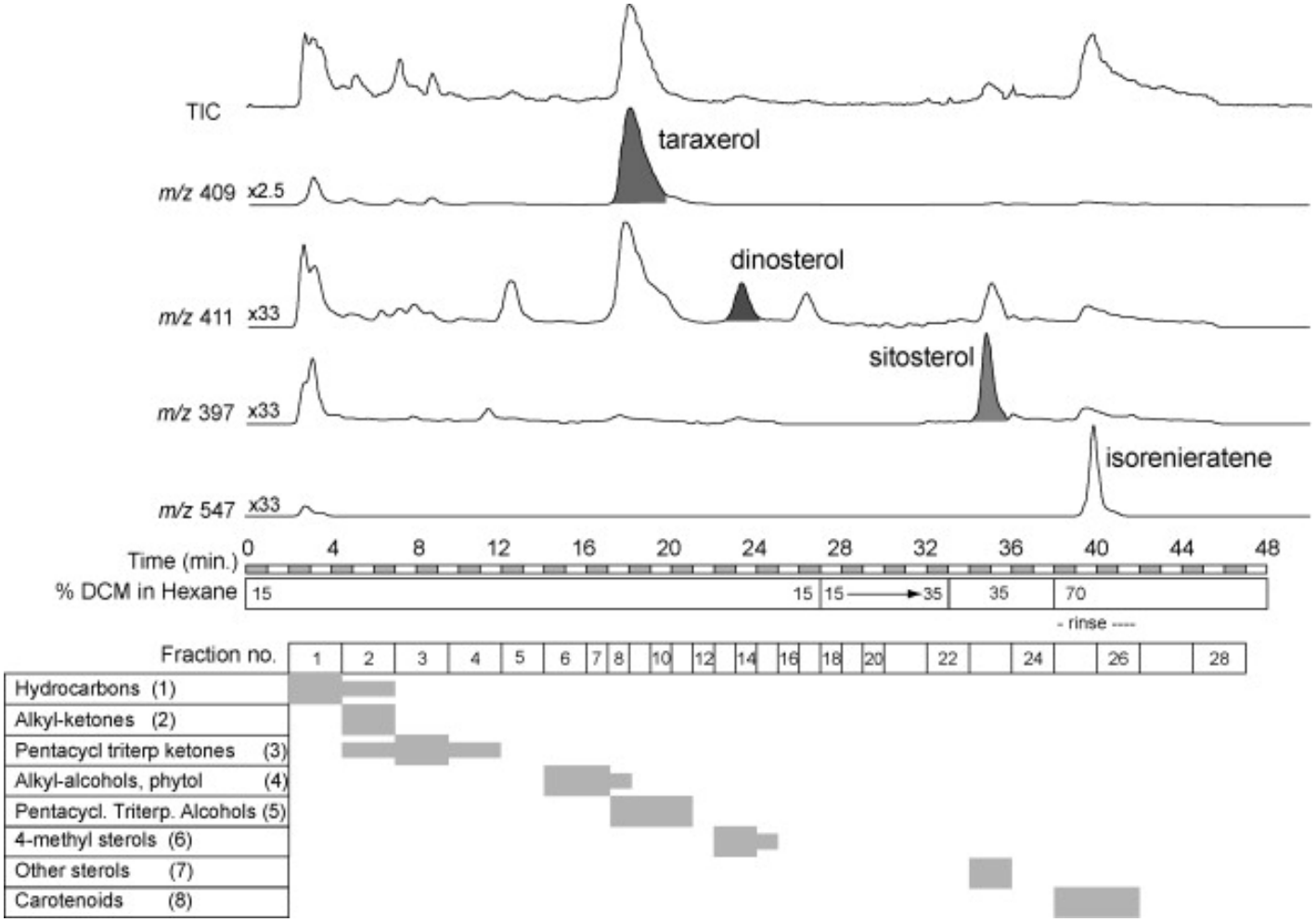
HPLC
No theoretical upper limit

GC
Typically < 500 amu

Example of sterol fraction

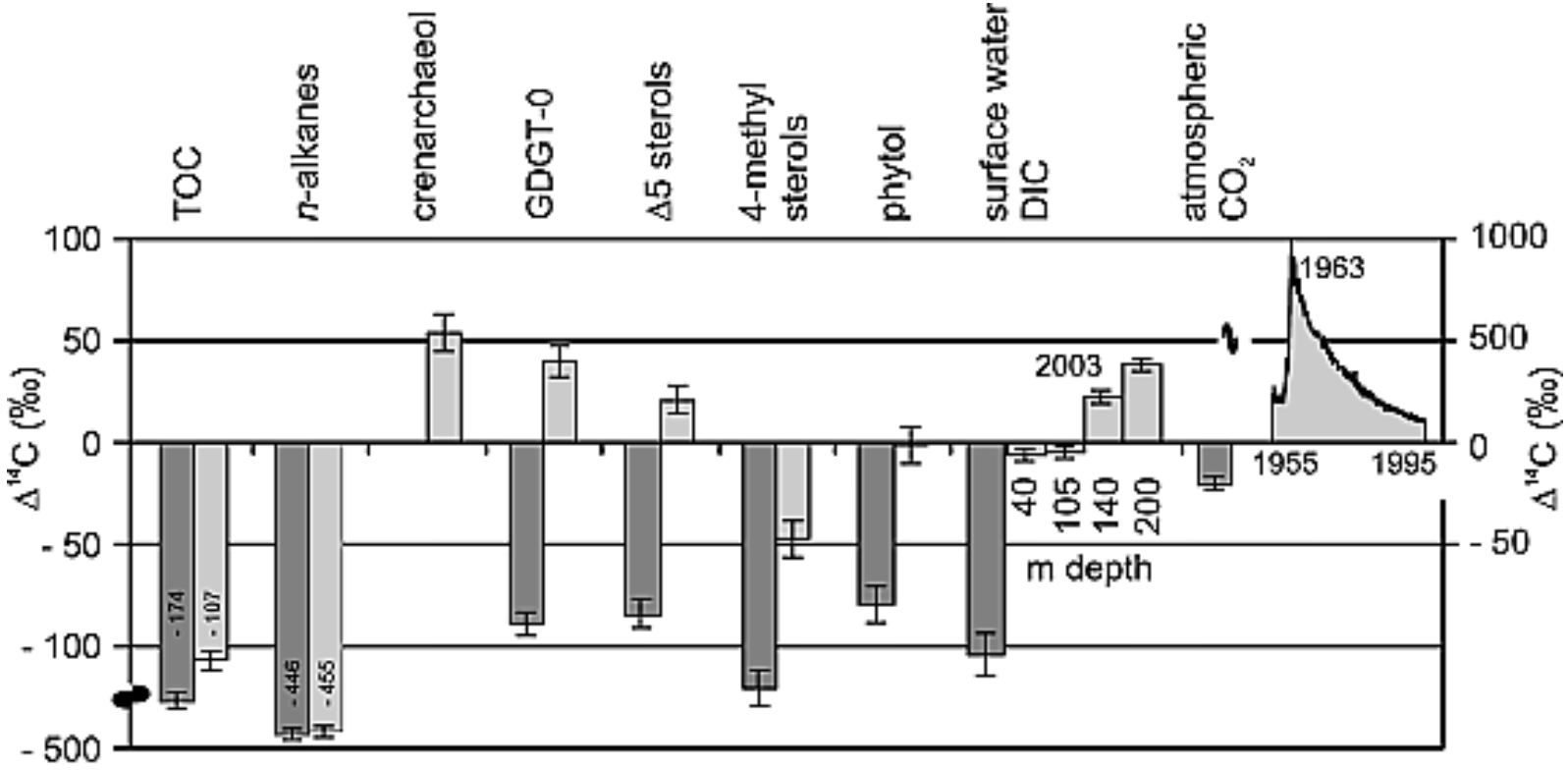


Example of sterol fraction run on HPLC



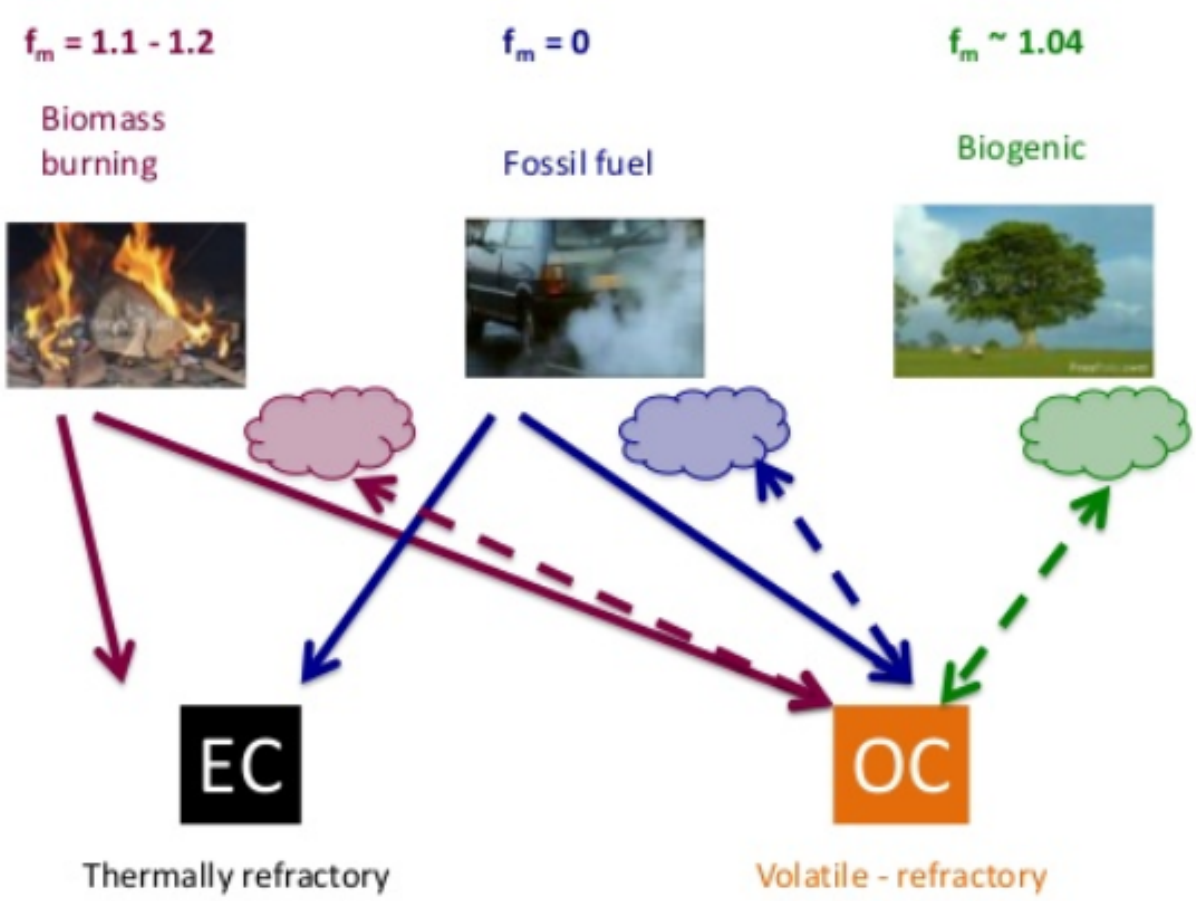
Smittenberg et al., 2007, Journal of chromatography A

Example of sterol fraction run on HPLC for "CSRA"

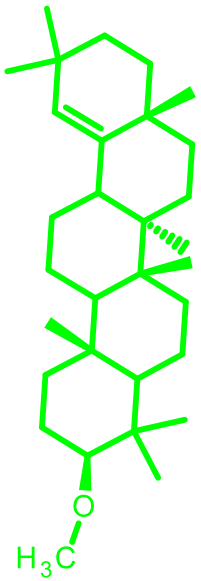


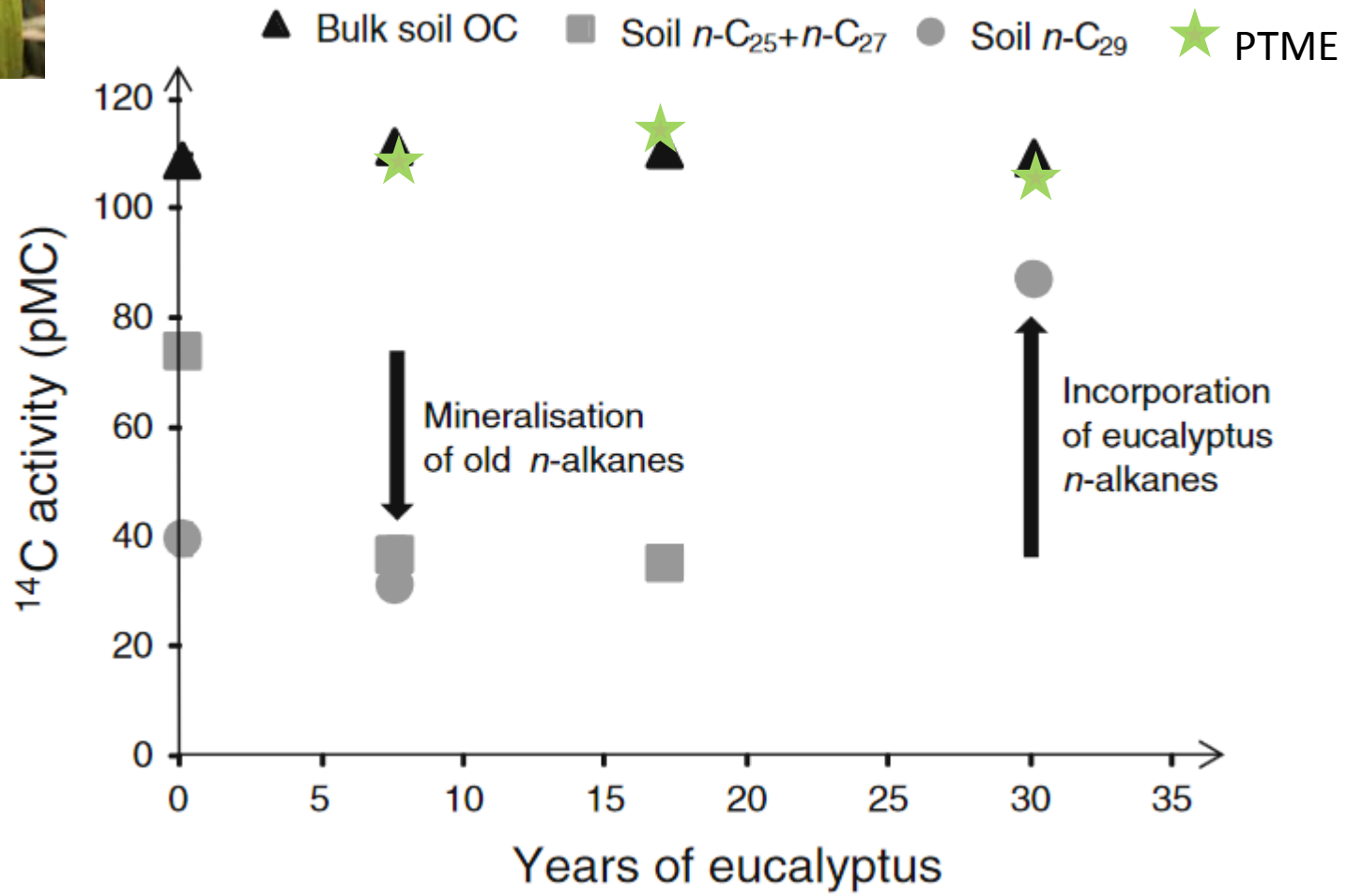
Smittenberg et al., 2006 Paleooceanography

CSRA of aerosol used for source appointment



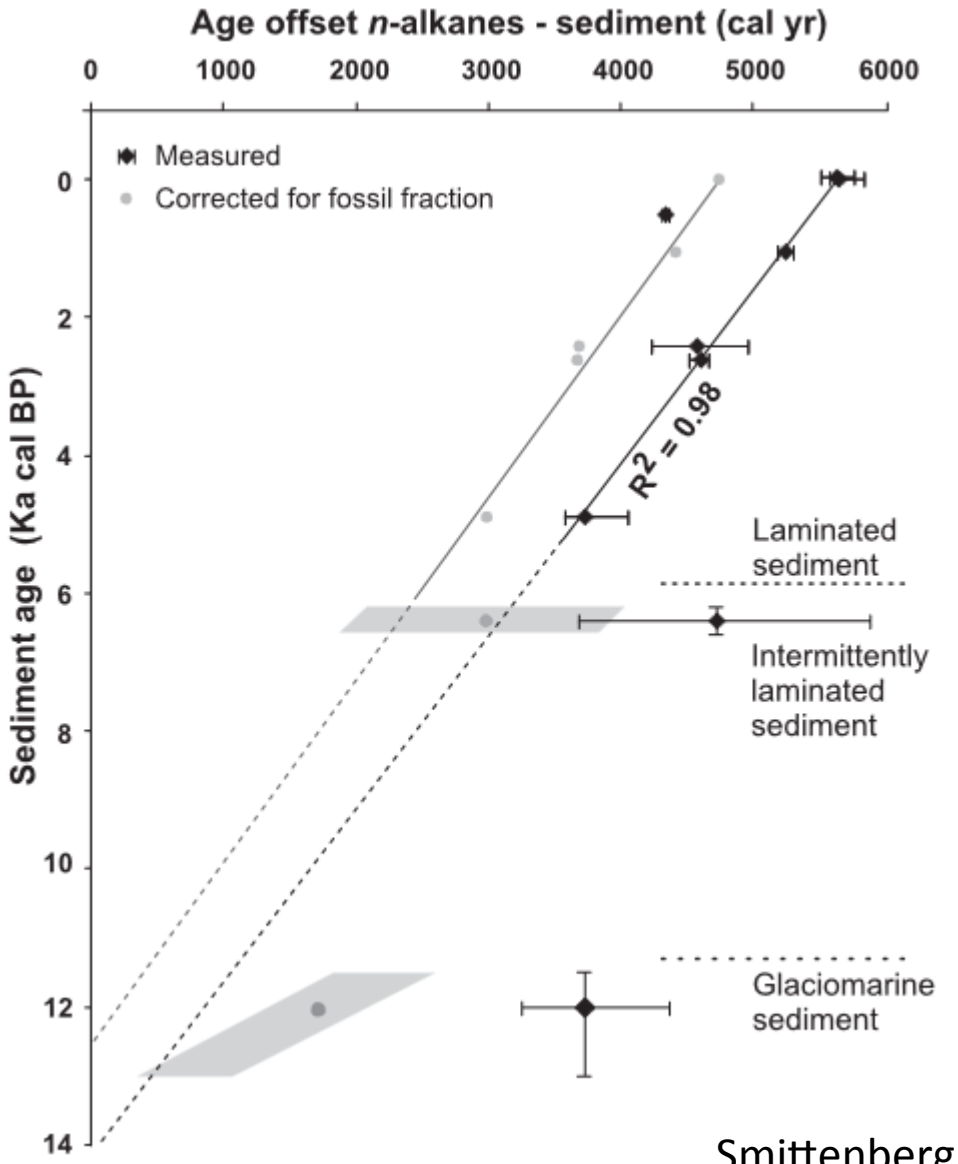
Different compound stabilization in savanna soil





Mendez-Millan et al., 2014 Biogeochemistry

Stabilization of wax *n*-alkane in sediments



Smittenberg et al., 2006 Science

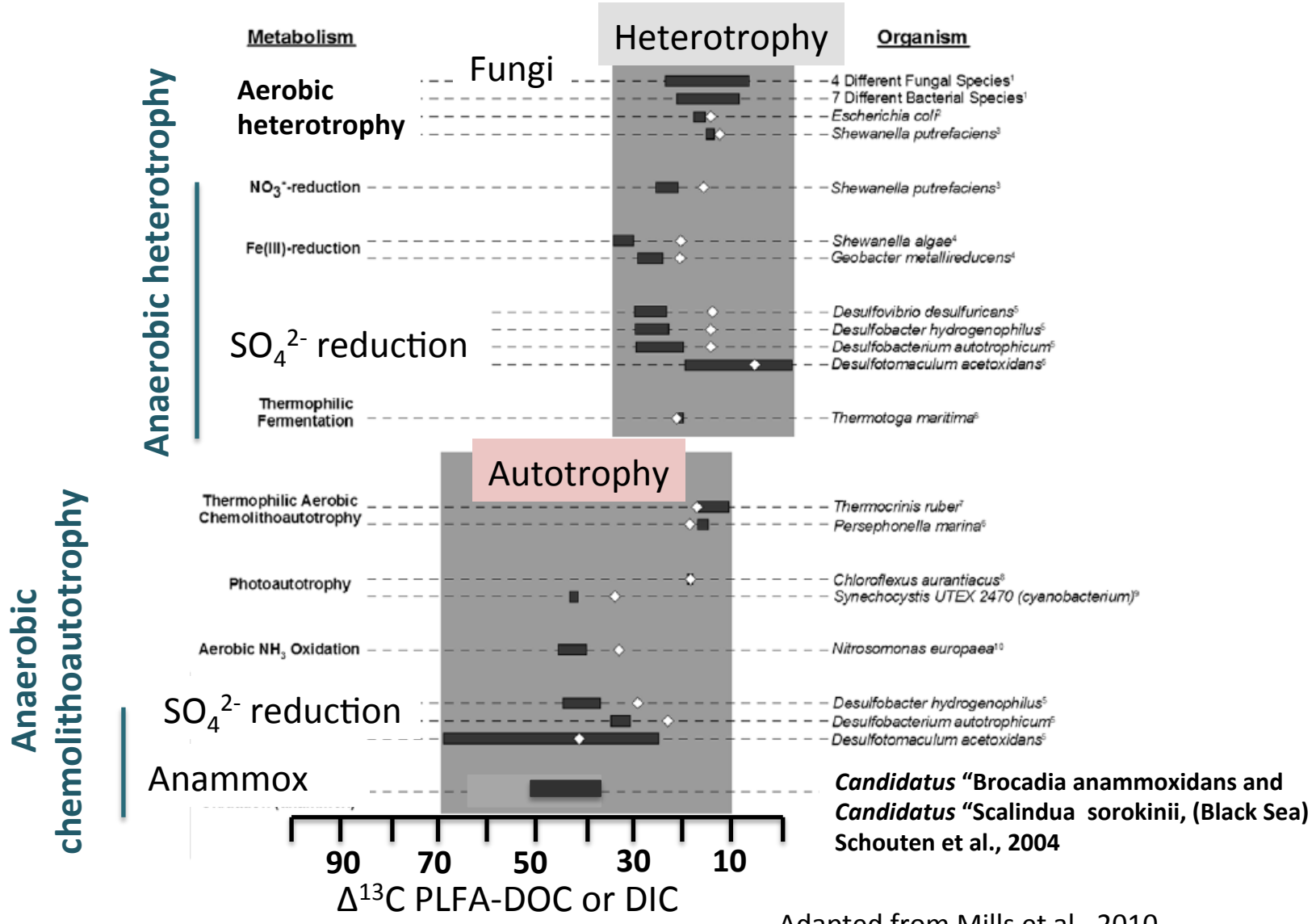
A EXAMPLE OF PLFA CSRA in groundwater

to help to define

- **the source of carbon used by an organism and the apparent age of the reservoir providing this source.**

- **the metabolic pathways of organisms**

PLFAs $\delta^{13}C$ values have a lot of overlap

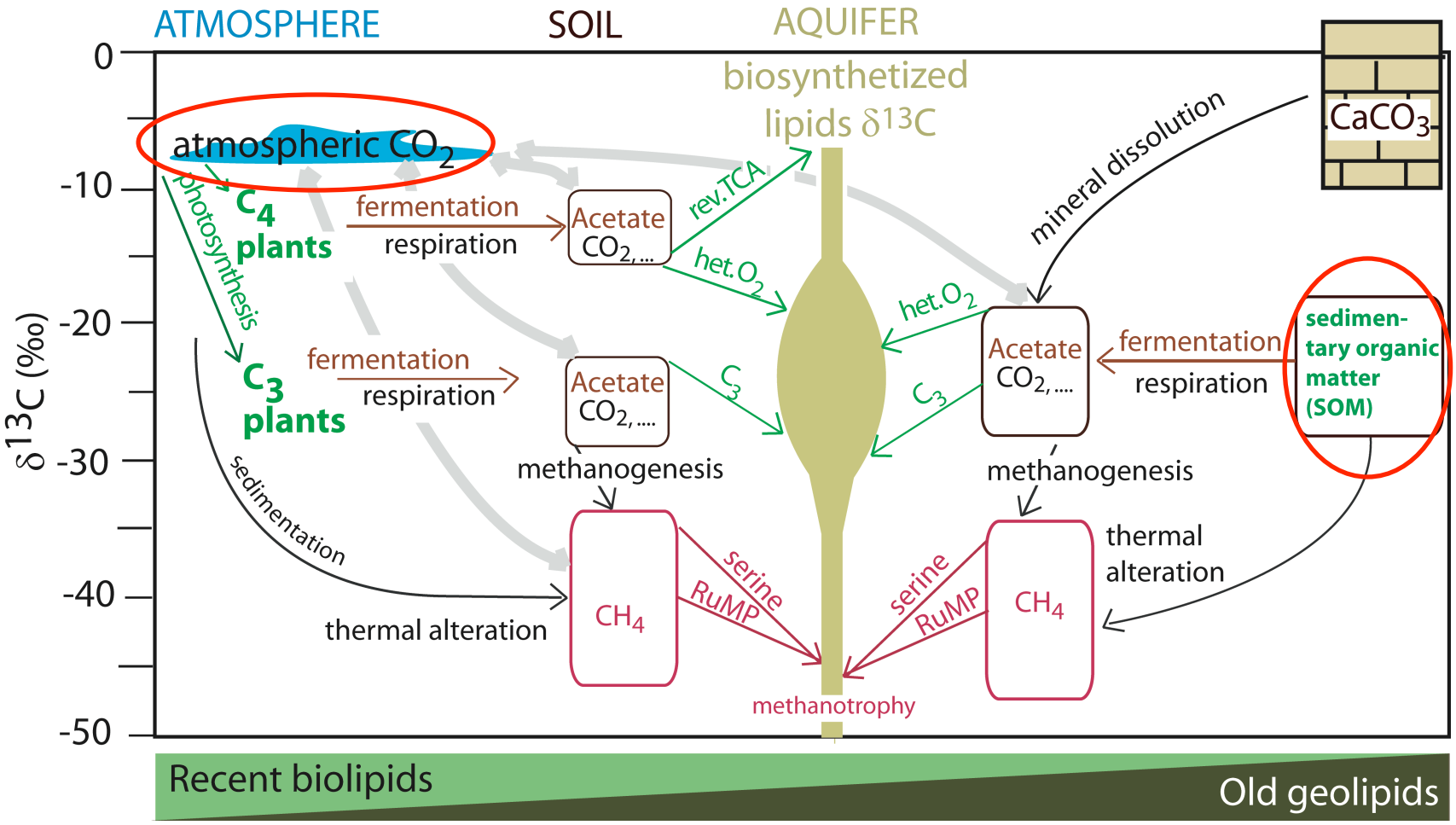


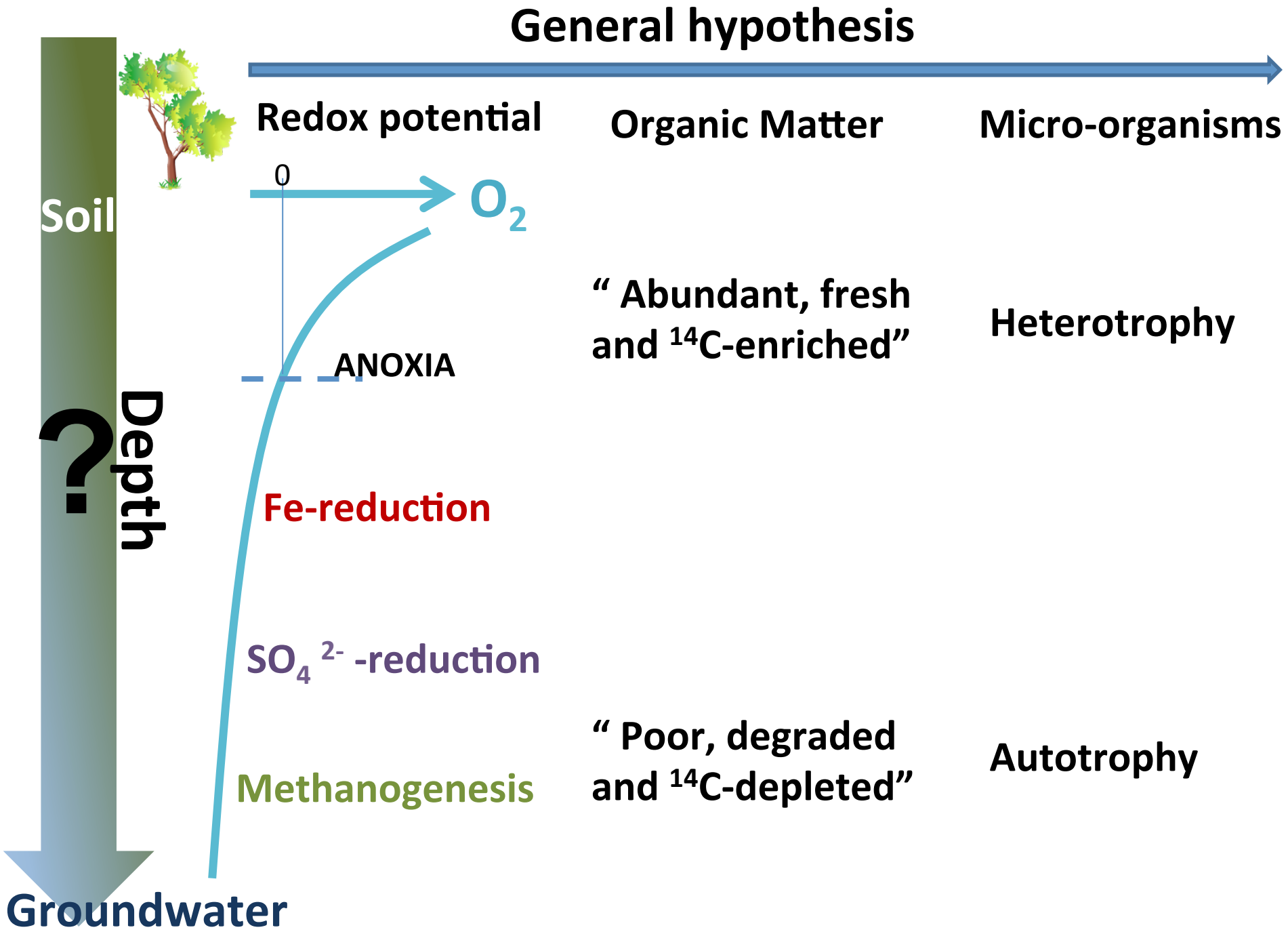
***Candidatus* "Brocadia anammoxidans and *Candidatus* "Scalindua sorokinii, (Black Sea) Schouten et al., 2004**

Adapted from Mills et al., 2010

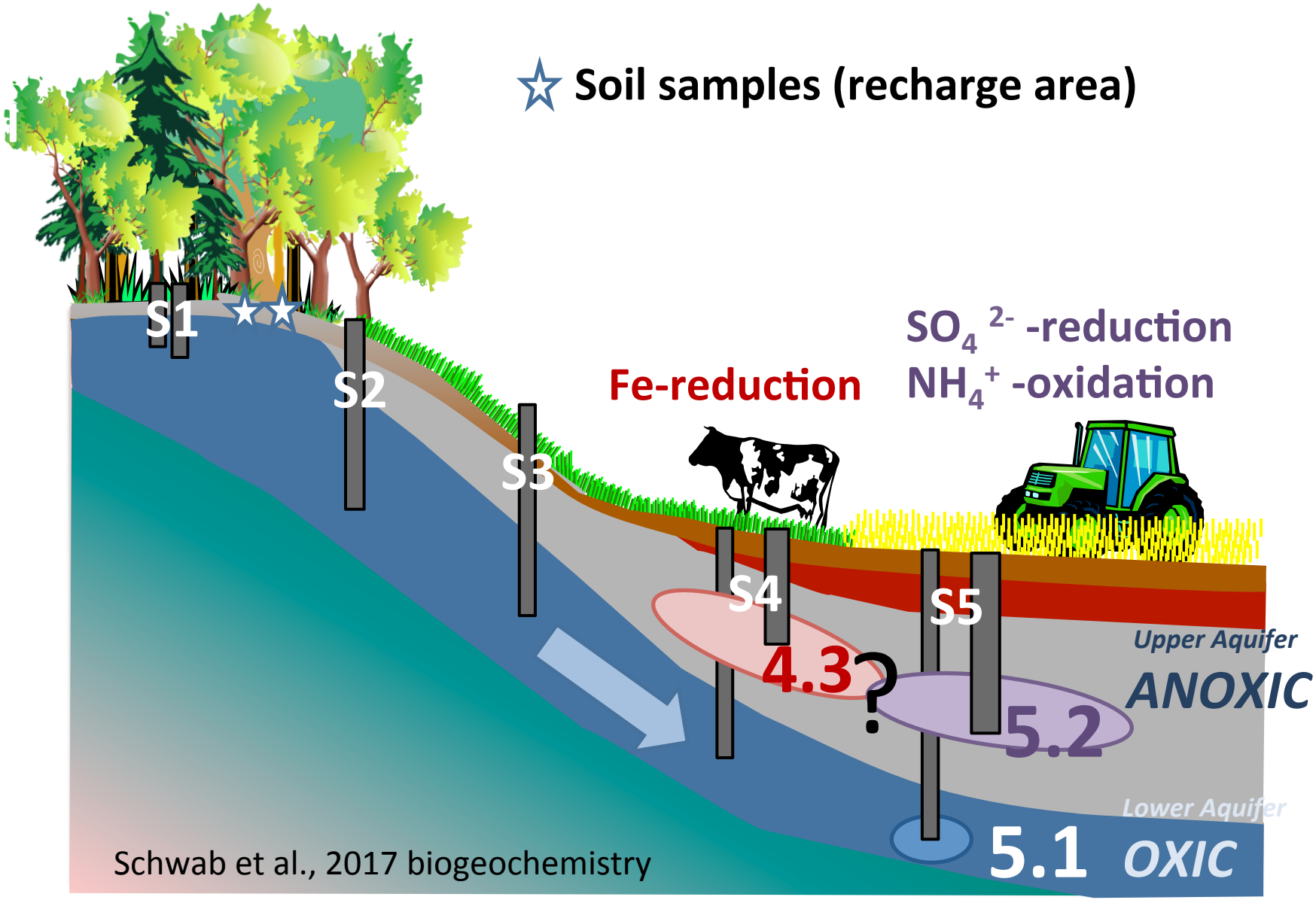
PLFAs ^{14}C values help to definite the C source

Mainly 3 organic pools in groundwater




General hypothesis
Redox potential
Organic Matter
Micro-organisms
Soil
0
O_2
ANOXIA
" Abundant, fresh and ^{14}C-enriched"
Heterotrophy
?
Depth
Fe-reduction
SO_4^{2-}-reduction
" Poor, degraded and ^{14}C-depleted"
Autotrophy
Methanogenesis
Groundwater

Condition of the sampling sites

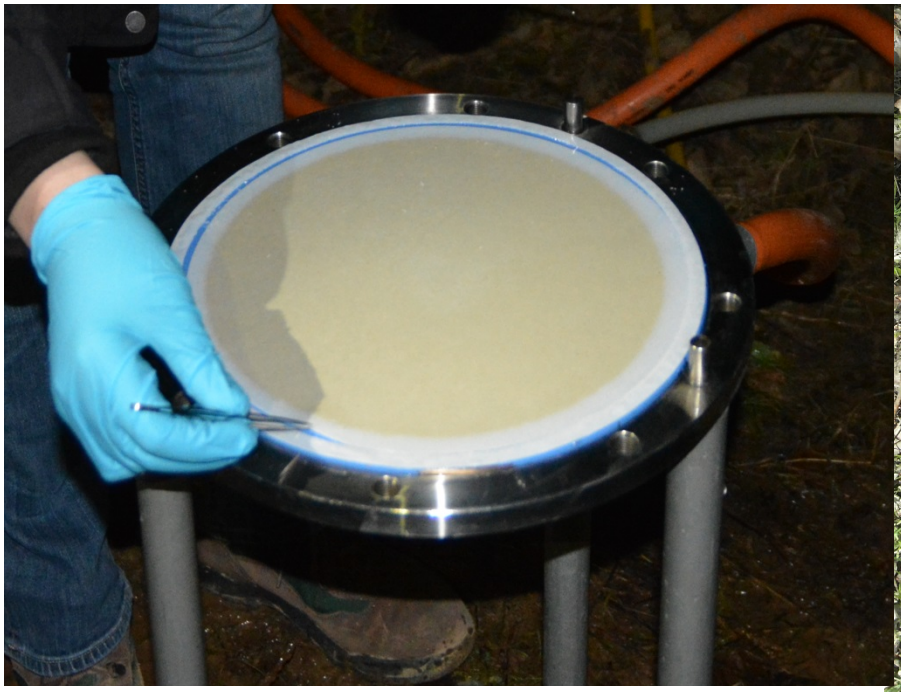


Sampling

View borehole
(ca. 10´000L)

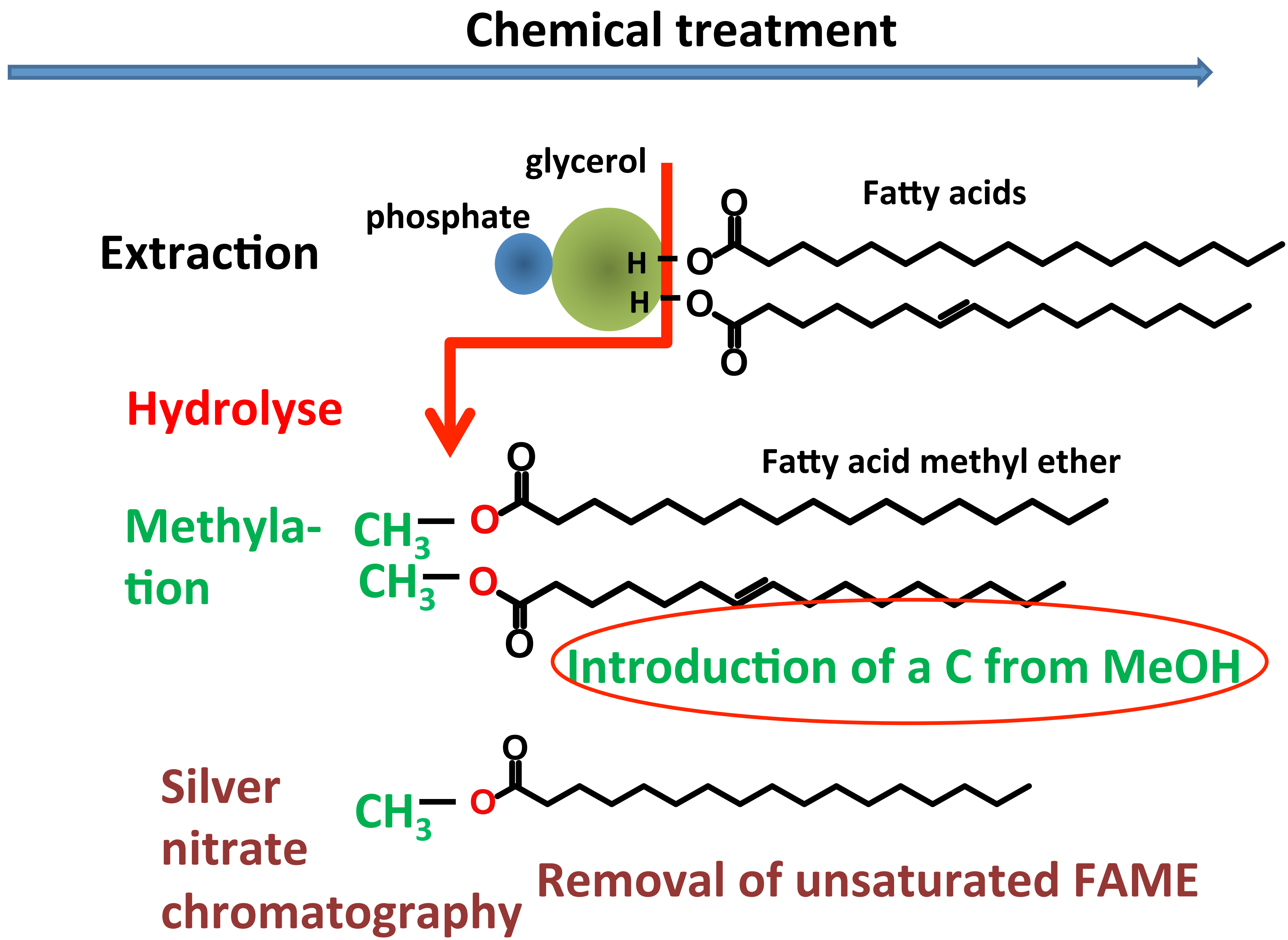
Filtration system
pre-combusted (5h 500°C)
glass fiber membrane
Ø 293 mm, 0.3 µm

Sampling



H5.2 sulfate-rich well
(ca. 10´000L)

H4.3 Iron-rich well
(ca. 10´000L)


Chemical treatment
Extraction
glycerol
phosphate
Fatty acids
H
O
Hydrolyse
Fatty acid methyl ether
Methyla-
tion
CH_3
Introduction of a C from MeOH
Silver
nitrate
chromatography
Removal of unsaturated FAME

Semi pre-HPLC

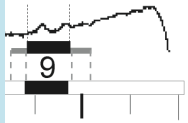
Eluting peaks in a C_{18} HPLC column

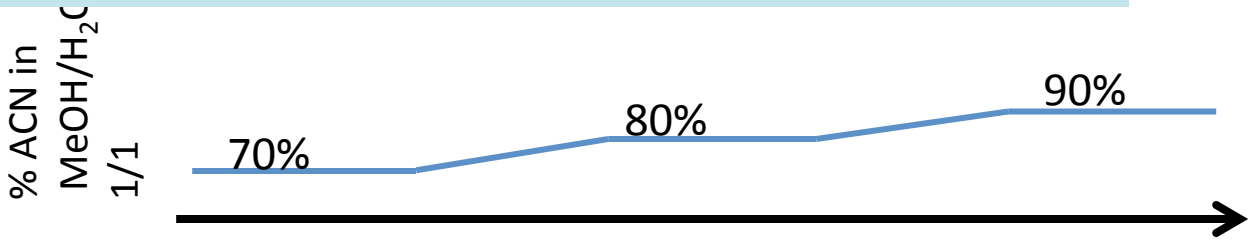
(150 x 4.6 mm, 3 µm)

Facts:

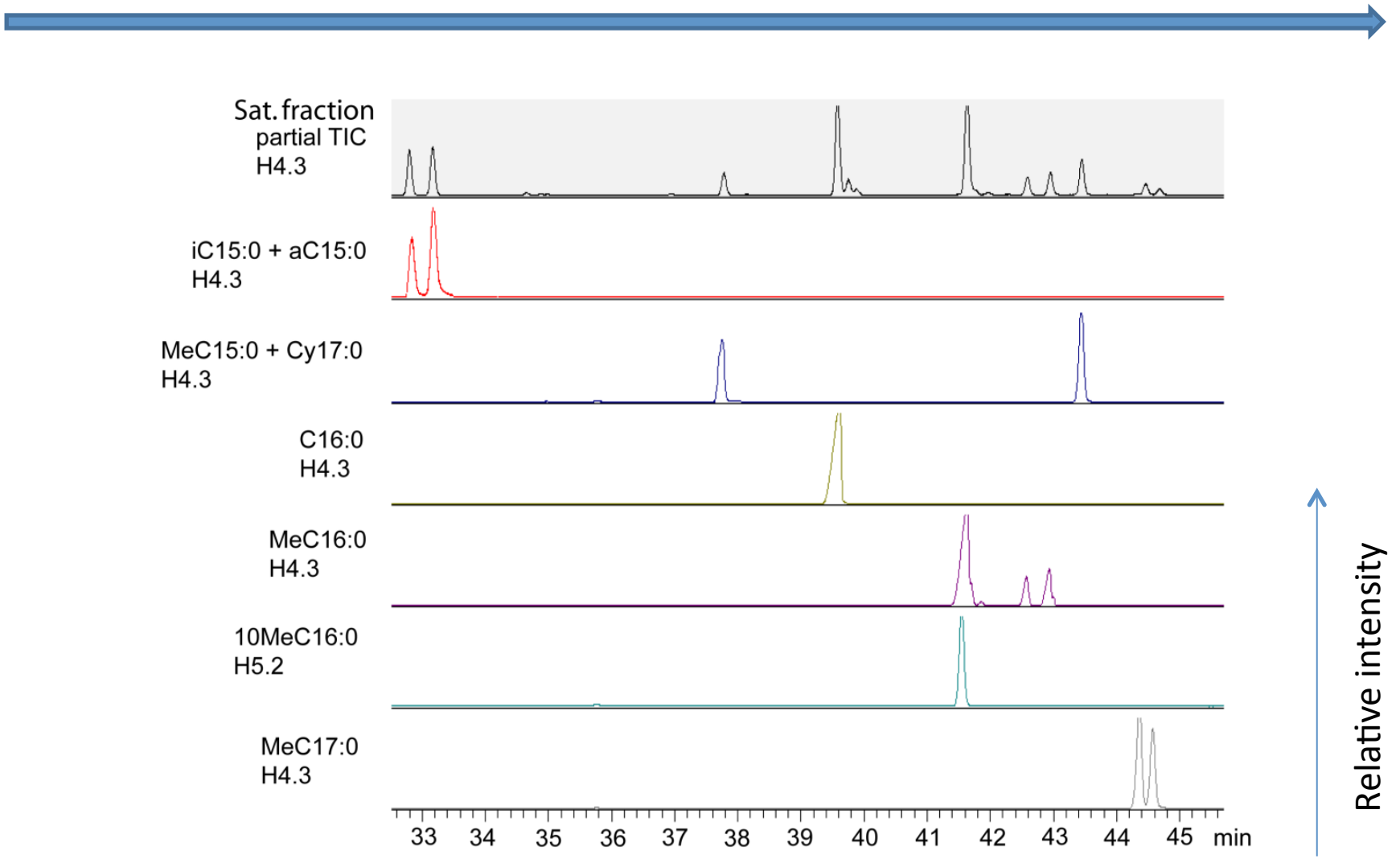
- **Between 5 up to 10 µg of a single compound per injection**
- **Need between 15 and 10 injections for collecting ca. 100 µg**
- **Can collected many different compounds per injection**

compoun

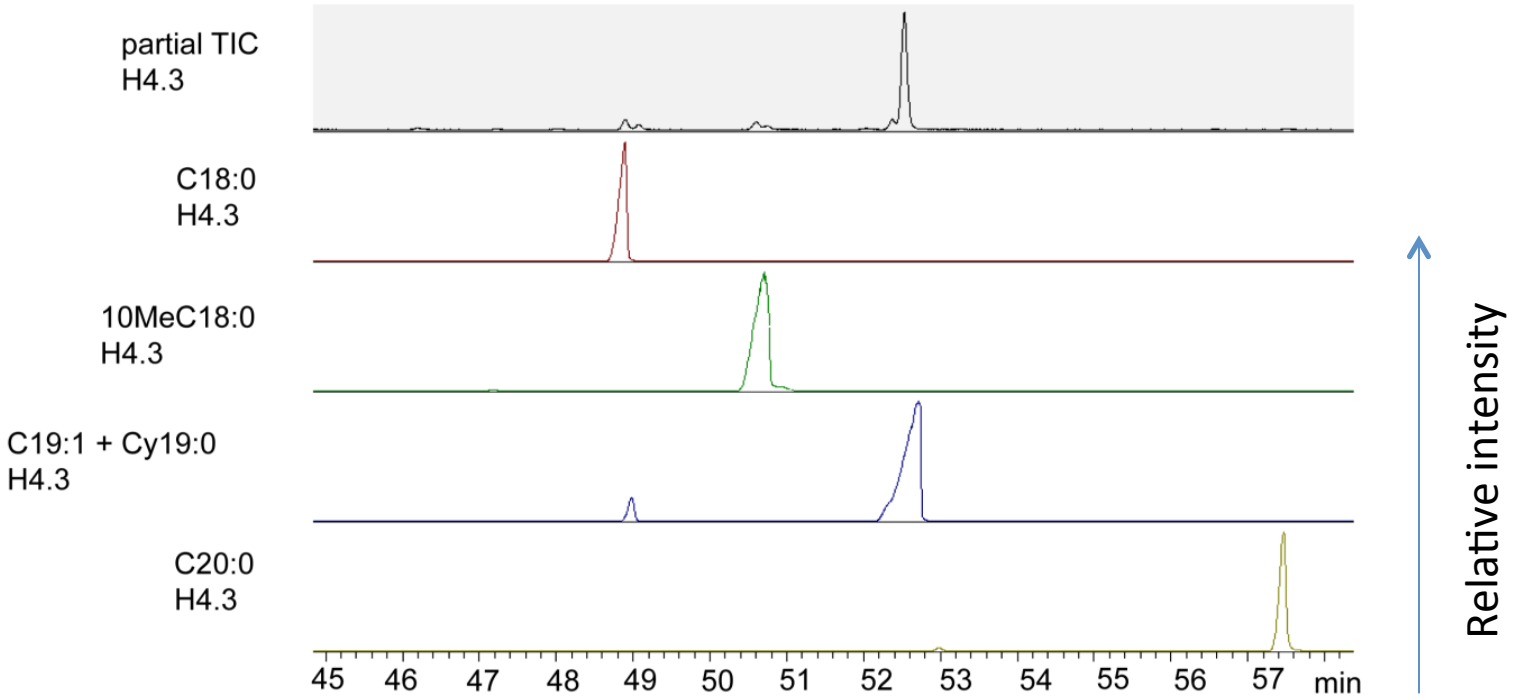




TIC (GC-MS) of the different collected HPLC fractions

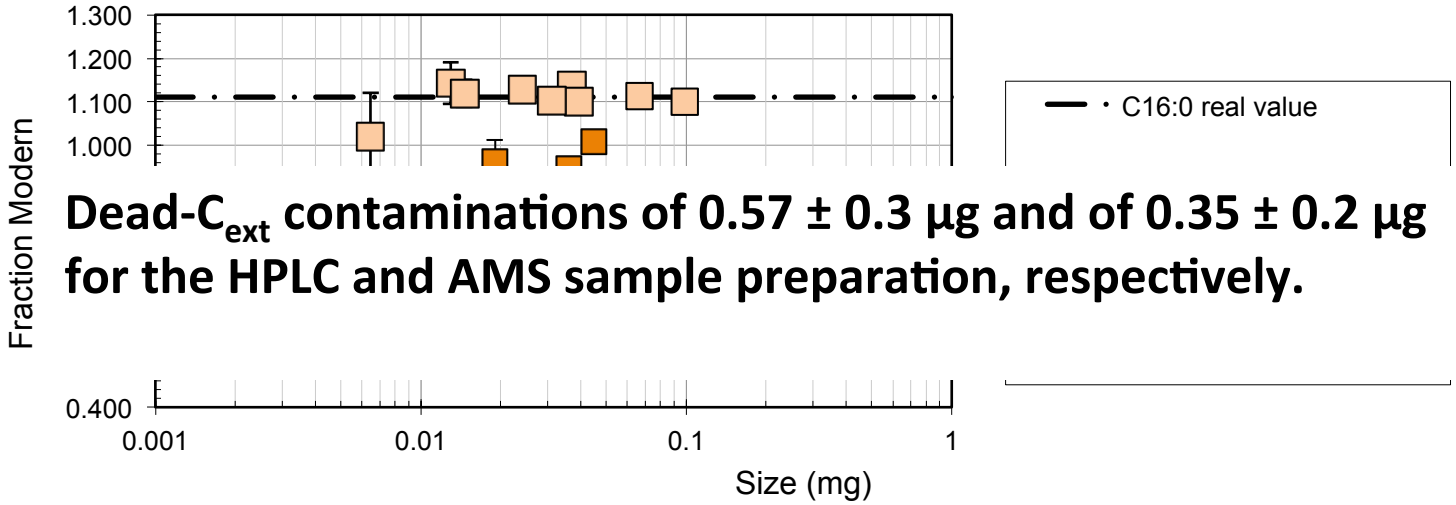


TIC (GC-MS) of the different collected HPLC fractions



HPLC column contamination

C16:0 FAME standard: -132 ± 3‰

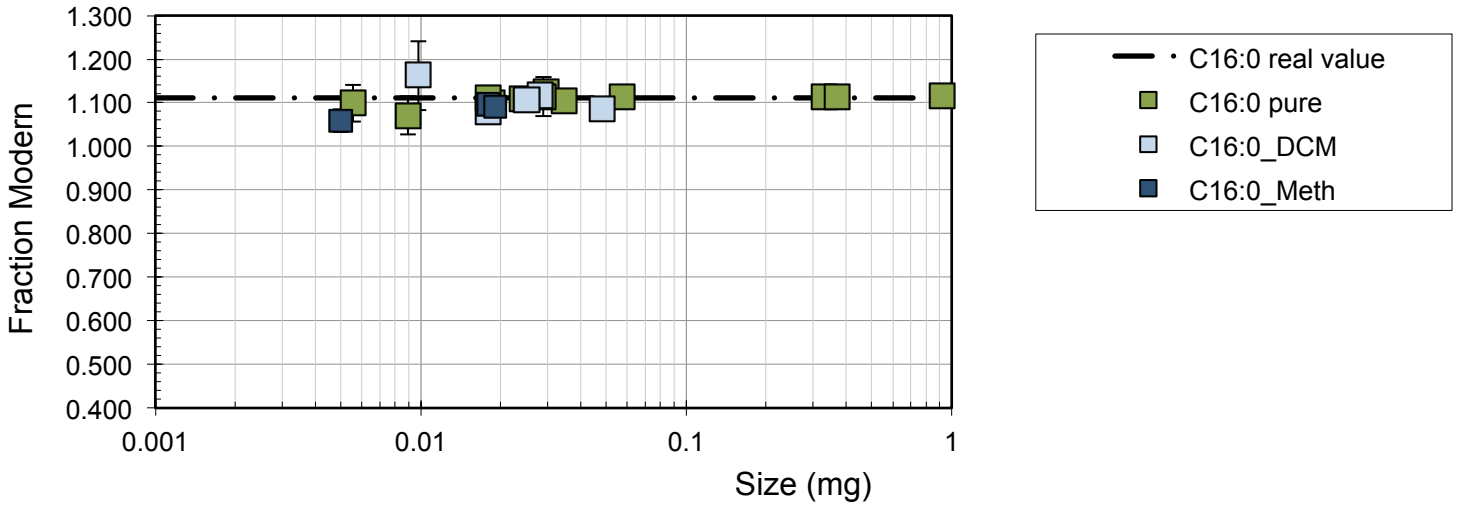


Dead-C_{ext} contaminations of 0.57 ± 0.3 µg and of 0.35 ± 0.2 µg for the HPLC and AMS sample preparation, respectively.

Phenanthrene standard: -997 ± 1‰

Modern C_{ext} contamination was 0.40 ± 0.20 µg for AMS sample preparation only.

Solvent contamination



- Each system will be distinct in term of Cext, therefore each user needs to evaluate the Cex and Fmex values specific for their system

- When considering CSRA applications, one must consider the magnitude of uncertainty required to provide useful information about the system being studied.

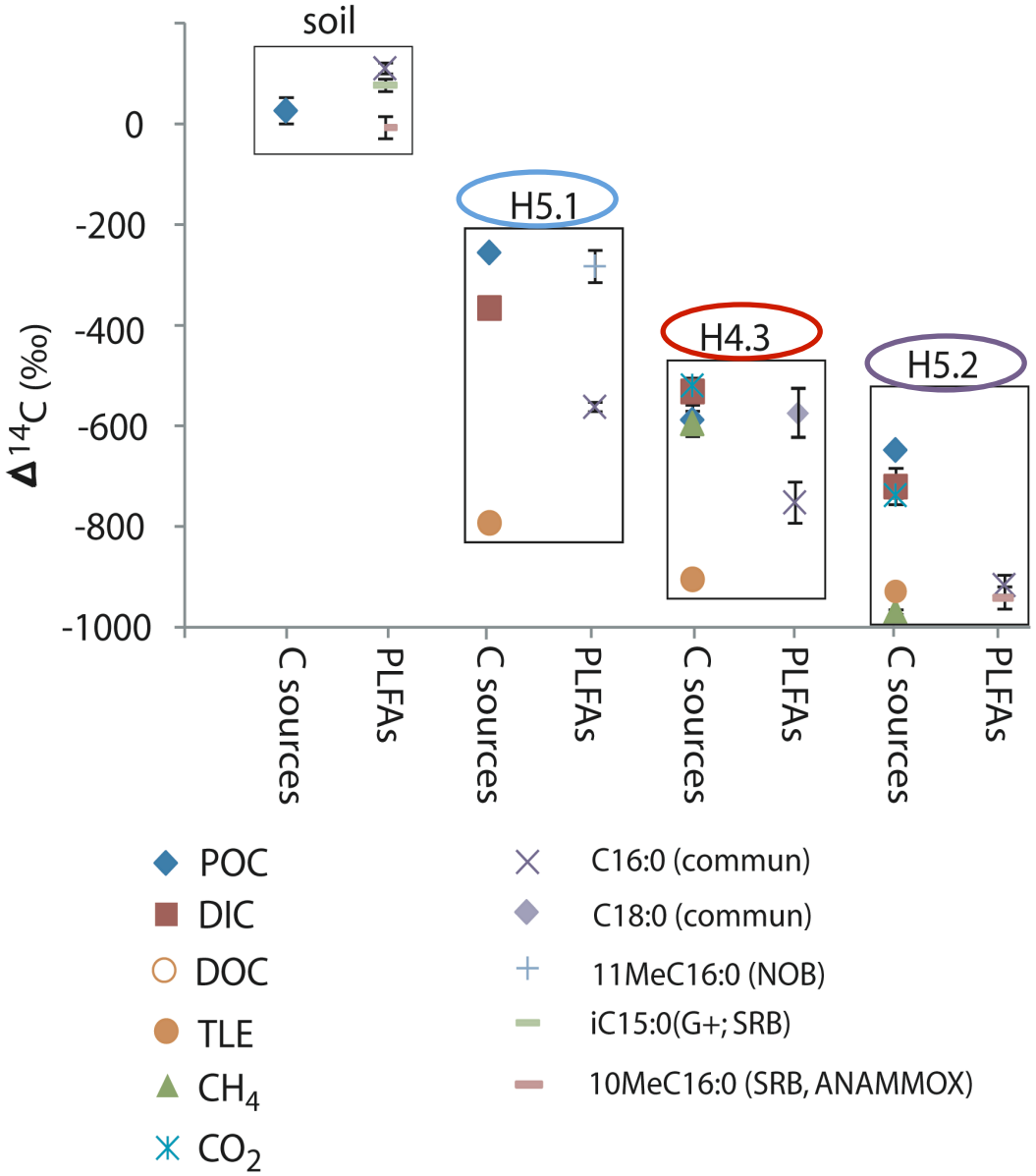
Mean recoveries

Table 2: Compound recovery in µC measured after the different purification and extraction steps

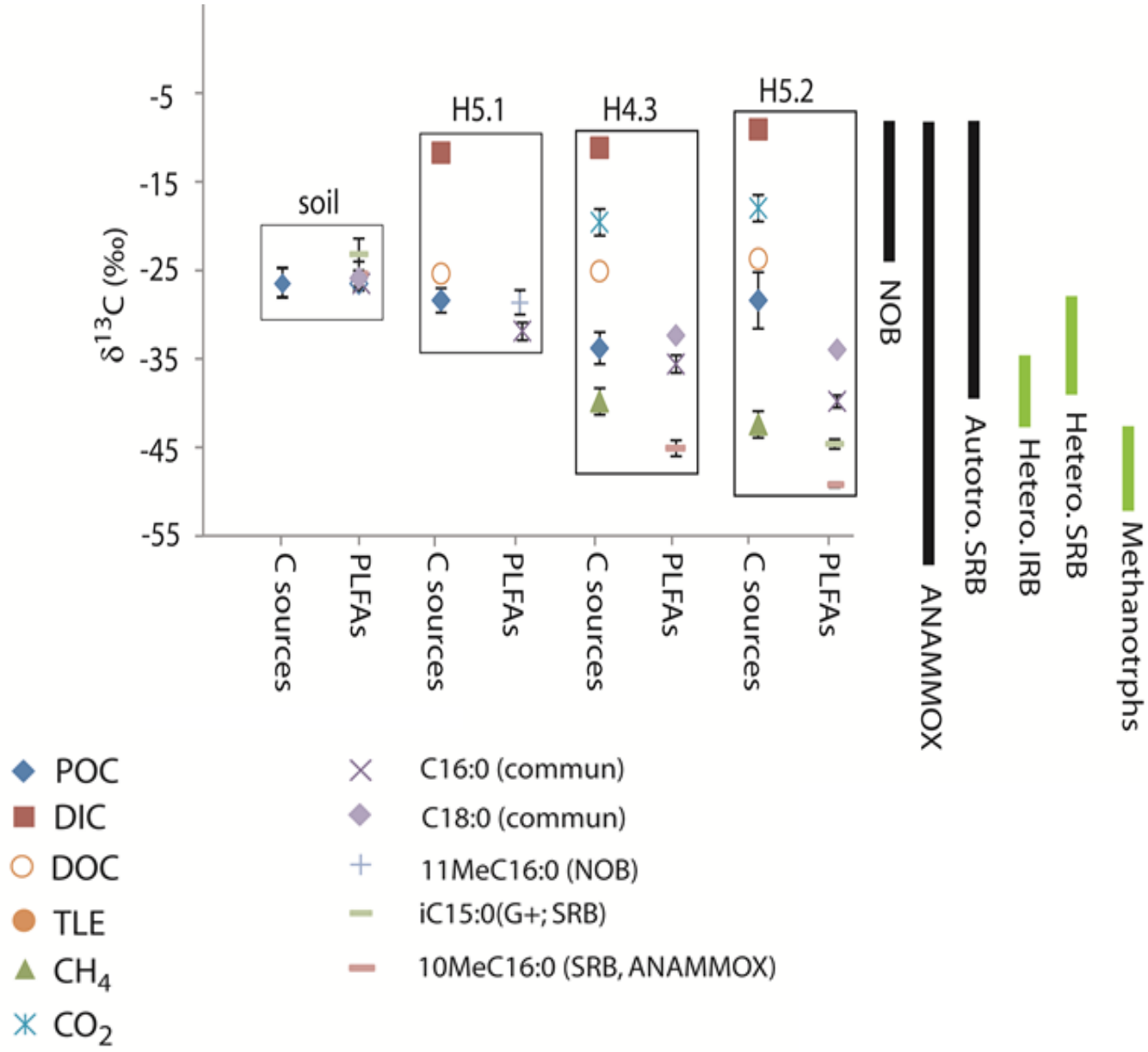
% recovery extraction + methylation + NH_2 column	% recovery of Ag-impregnated SCX column	% recovery of HPLC purification	% recovery of solvent evaporation and combustion	Total recovery
89±10 (2)	88±4 (9)	83±6 (9)	71±13 (9)	46±16 (9)

Larger compound lost during solvent evaporation or/and in vacuum line

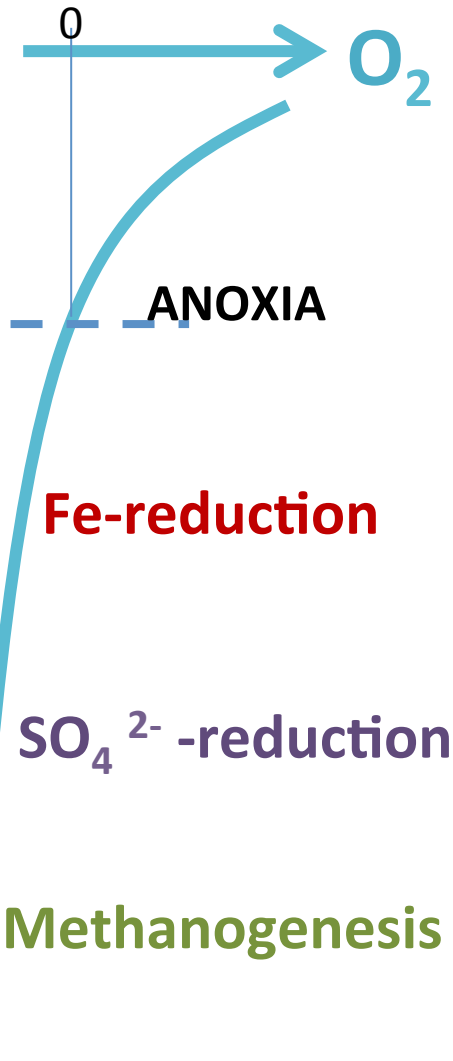
Results and implications



Results and implications



Conclusion



Redox potential	Organic Matter	Micro-organisms
O_2	" Abundant, fresh and ^{14}C-enriched"	Heterotrophy
SO_4^{2-}-reduction	" Poor, degraded and ^{14}C-depleted"	Heterotrophy

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Thank you for your attention

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