Environmental Signals from ‘Biochemical Dustbins’

Richard Evershed
Organic Geochemistry Unit
Bristol Biogeochemistry Research Centre
School of Chemistry
Environmental ‘Dustbins’ for Biochemical Products

- Plant matter
- Animal matter
- Gaseous products
- Mineral products
- Microbial matter
- Atmosphere
- Water
- Lake sediments
- Ocean sediments
- Soil
- Chemical and microbial processing (decay)
- Geological deposits
- Archaeological remains
- Soils and peats
- Recycling

Living world

Dead organic matter

Return to the living world
Analytical chemistry of molecules past and present

BIOMOLECULAR PALAEONTOLOGY
Fossilisation processes and palaeoenvironmental information

ANALYTICAL CHEMISTRY
Molecular and stable isotopic compositions

BIOGEOCHEMISTRY
Role of microbes and invertebrates in soil organic matter cycling

ORGANIC GEOCHEMISTRY
Reconstructing past climate and environments (peat bogs and lakes)

BIOMOLECULAR ARCHAEOLOGY
Reconstructing human activity in the past
Biomarker compounds

Molecules that can be linked to biological sources due to their characteristic structures

Plant leaf wax biomarker

Tree resin biomarker

Bacterial fatty acid biomarker
Need to determine either one or a few compounds from materials containing many thousands of substances.
Analytical Chemistry: ‘Needle from a Haystack’

Biochemically complex samples
100,000s of compounds

Solvent extraction

Lipid extract
1,000s of compounds

Separation

Separation

Separation

Fraction
10s of compounds

Gas chromatography
Do we have biomarkers?
How much do we have?

GC/mass spectrometry
What are the structures of the biomarkers?

GC/isotope ratio/MS
What are the stable isotope values of the biomarkers?
State-of-the-art instrumentation vital for this type of work
Gas chromatography based analytical methods

Gas chromatography
Separates compounds according to differences in boiling point and polarity

Intensity
Retention time

1400°C
D/H + H₂
Isotope ratio mass spectrometry
δD values

O₂/Cu/Pt 850°C
13CO₂ + 12CO₂
Isotope ratio mass spectrometry
δ13C values

Organic mass spectrometry
Mass spectra
Structures
Palaeoclimate reconstruction
‘Using the past to predict the future’
‘Using the past to predict the future’

Ocean sediments

Tree rings

Ice cores
Using the past to predict the future: Peat bogs as archives of past climate

Ombrotrophnic mire formation

Atmosphere the only source of nutrients

Plants growing on surface highly sensitive to climate change
Peat bogs as archives of past climate.
Identification of biomarkers by analysis of modern plants

Sedge biomarker

Sedge fossil

\( \text{C}_{29} \)

(Prefer drier conditions)

Sphagnum moss biomarker

Sphagnum fossil

\( \text{C}_{23} \)

(Prefer wetter conditions)
GC analysis of peat $n$-alkanes

Sphagnum moss biomarker

Sedge biomarker

Bacterial biomarker

Retention time
n-Alkanes recording the changes in plants forming peat bogs in the past.

Changing vegetation

Peat core

Past

Present day

Increasing bog surface wetness

210Pb/14C

0.01

1 10

n-C23

Sphagnum (%)

0 100

0.01

0.1

1

10 Log [C23/31]

Calendar years (A.D.)

1770 1820 1870 1920 1970

n-Alkanes recording the changes in plants forming peat bogs in the past.
Effect of temperature on $^2$H/$^1$H ratio ($\delta D$)

The $^2$H/$^1$H content at higher latitudes (>30°) varies seasonally with a maximum in the summer and a minimum in winter.

**Summer:** high $\delta D$

**Winter:** low $\delta D$

Hence, $\delta D$ of plant biochemical should reflect differences in temperature during growing season.
n-Alkane $\delta D$ values correlate with recorded temperature for the past 200 years
The ACCROTEL Project

Generating calibrated records of rainfall and temperature change from peat bogs across Europe using biomarkers and a range of other indicators
Biogeochemistry of the methane cycle:

‘Stable isotope fishing for methane eating bacteria’
Methane (CH$_4$) and global warming

- Global CH$_4$ concentrations have been increasing for the past 300 years reaching a global mean of 2 ppmv

\[ \text{CH}_4 \text{ (ppb)} \]

\[ \begin{array}{cccccc}
1000 & 1200 & 1400 & 1600 & 1800 & 2000 \\
600 & 800 & 1000 & 1200 & 1400 & 1600 \\
\end{array} \]

Etheridge *et al.* (1998)

- CH$_4$ contributes (mole for mole) 26 times more to climate change than CO$_2$

- During the past century CH$_4$ methane has contributed 15-25% to global warming
Sources and sinks of methane

- **Sources of CH₄**
  - Wetlands
  - Agriculture
  - Landfill Sites
  - Energy production and use
  - Natural gas venting
  - Biomass burning
  - Domestic Sewage

- **Sinks of CH₄**
  - Chemical reactions in the atmosphere with OH
  - *Soils via methane oxidising bacteria* (methanotrophic bacteria: methane eating bacteria)

- An enhanced understanding of the methanotrophic bacteria would help in managing methane emissions
  
  e.g. forest management, fertilizer effects, etc.
Methane oxidising bacteria – methanotrophic bacteria

- Two main groups:

*Low affinity* methanotrophs oxidise methane at high concentrations and have been isolated and characterised

\[
\text{CH}_4 \rightarrow \text{CH}_3\text{OH} \rightarrow \text{HCHO} \rightarrow \text{HCO}_2\text{H} \rightarrow \text{CO}_2
\]

Divided into three main groups
- Type I: C\textsubscript{16} fatty acids
- Type II: C\textsubscript{18} fatty acids
- Type X: mixture of characteristics

• *High affinity* methanotrophs oxidise methane at low concentrations but cannot be isolated and cultured

• Major problem - these are the soil methane sink!
Culture independent methods: 
**Phospholipid fatty acid (PLFA) analysis of soil microbes**

- **Phospholipids are major components of cell walls**
- **Phospholipids short-lived upon cell death**
- **PLFAs represent living microbes**

**Phospholipid**

Chemical release

**Phospholipid fatty acids (PLFAs)**
• Wide range of microbial groups represented but impossible to say which PLFAs come from methanotrophs
‘Stable isotope fishing for methane eating bacteria’
Selective $^{13}$C-labelling of subset of microbial population

Highly complex soil microbial community

$^{13}$CH$_4$
Incubate

Only bacteria consuming $^{13}$CH$_4$ become $^{13}$C-labelled

- Challenge: detection of very low concentrations of $^{13}$C-labelled PLFA amongst 10,000 x the concentration of unlabelled PLFAs
Soil incubations

Flow through chamber

Synthetic air + $^{13}$CH$_4$

Chamber flushed every 24 h

Exposure of soils to different concentrations of $^{13}$CH$_4$: high and low affinity methanotrophs

- Analyse PLFAs by GC-IRMS to locate $^{13}$C-label

- Only the PLFAs from the methanotrophic bacteria take up $^{13}$CH$_4$

- Dominance of 18:1 PLFA indicates Type II methanotrophic bacteria oxidising methane at both high and low concentrations

- Concentrations of $^{13}$C-labelled PLFAs indicates methanotrophs are ca. 0.01% of all soil bacteria

Effects of low input farming in the Brecon Beacons, South Wales

- Run by IGER (Institute of Grassland & Environmental Research)
- Long term study: 6 different soil treatments over the past 14 years
- Wider investigation the impact of grazing and other agricultural practices on uplands

What is the impact of the various treatments on methanotrophic bacteria?
Effect of a common fertilizer on soil methanotrophs

Total soil bacterial PLFAs

N Ca P K fertilizer

Increased soil bacteria

13C-labelled PLFA µg g⁻¹

Grazed

N Ca P K fertilizer

Decreased methanotrophic bacteria

13C-labelled PLFA µg g⁻¹

Grazed

Phospholipid fatty acid (PLFA)
Reconstructing human activity in the past

‘Molecules that time forgot’
Archaeological Biomarkers

Ancient biomolecules that carry information concerning human activity in the past

Can be any class of compound preserved in organic residues at archaeological sites, e.g. DNA, protein, lipids, etc.
Bronze Age soil

Resins, tars, pitches and bitumens

Roman cosmetic

Animal mummy

Plant remains

Skeletal remains

Bog body
GC/MS of Wood Tar From King Henry VIII’s Flagship

Abietic acid

Pimaric acid

Heating

CO₂H

CO₂Me
Archaeological Information retrievable from
Preserved Ancient Biomolecules

- Commodities
- Specific uses of artefacts
- Ancient technologies
- Diet
- Agriculture
- Hunting

- Origin of peoples
- Trade
- Cultural differences
- Ritual activities
- Dating
- Decay (taphonomy)
Abundant
Durable
Diagnostic
Preserve organic residues

Potsherds
Surface residues

Organic Residues in Archaeological Pottery

Absorbed residues
GC/MS analysis of lipids from a medieval cooking vessel

Partial gas chromatogram

Mass spectra
GC ‘fingerprints’

Modern cabbage leaf wax

Wild-type cabbage

Late Saxon/Medieval cooking vessel

Relative intensity

$\delta^{13}C - 35.8$

$\delta^{13}C - 35.4$

$\delta^{13}C - 34.8$

$\delta^{13}C - 34.6$

Time (min)

18 20 22 24
Degraded animal fats are identified by their characteristic distribution of mono-, di- and triacylglycerols and abundant saturated fatty acids.
Uses of Fats (and Oils) in Antiquity

- Essential dietary component
- Illuminants (lamp fuel and candles)
- Polishes and lubricants
- Art materials, e.g. binders
- Base for perfumes and ointments
- Embalming agents and religious commodities

- *Man would have actively recovered fat for these activities*

- *Often mixed fat with other commodities – pottery vessels would have been used in all these activities*
Classification of animal fats based on compound-specific $\delta^{13}C$ values of $C_{16:0}$ and $C_{18:0}$ fatty acids

Fats from modern animals

Dudd and Evershed Science (1998)
Isotopic difference between $C_{18:0}$ in ruminant carcass (adipose) and milk fats

Copley et al. PNAS (2003)
Dairying in Antiquity: The traditional evidence

- Relatively easy to identify domestic animals but much more difficult to establish exactly what they were used for

- Pictures

- Perforated vessels
  Said to be cheese strainers/presses?

- Archaeological bones
  Animals raised for dairy/meat/traction have different herd structures
Southern British sites investigated in milk project

Prehistoric Pottery

- 14 sites spanning early Neolithic to Iron Age
- ca. 1000 individual vessels analysed
Pottery from all sites show the presence of milk fat residues (\(\bullet\) = potsherd residue)

Dairying established when agriculture introduced into Britain 6000 years ago

Domestication

Secondary Products Revolution
Inc. exploitation of animals for meat and milk products

Model for the Emergence and Spread of Dairying Across Europe in the Neolithic

Arrival of pottery and agriculture (with dairying)

4,000 BC

7,000 BC

NW Europe
C and E Europe
SE Europe
Middle and Near East

Domestication
Inc. exploitation of animals for meat (and milk?)

Pottery production
Origins of dairying: sites and sherds

- >2,200 sherds selected from 23 sites

Animal fats detected in over 300 sherds
Dairy fats in SE European and Near Eastern Pottery

- Strongest evidence in NW Turkey
- Evidence for processing milk products in pottery at 6500 BC

**Non ruminant carcass fat**

**Ruminant carcass fat**

**Milk fat**
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