# Two examples of biomarker lipid applications to studies of carbon cycling in marine systems:

Part A: The determination of terrestrial organic matter in sediments *via* lignin oxidation products

by John I. Hedges & Co.

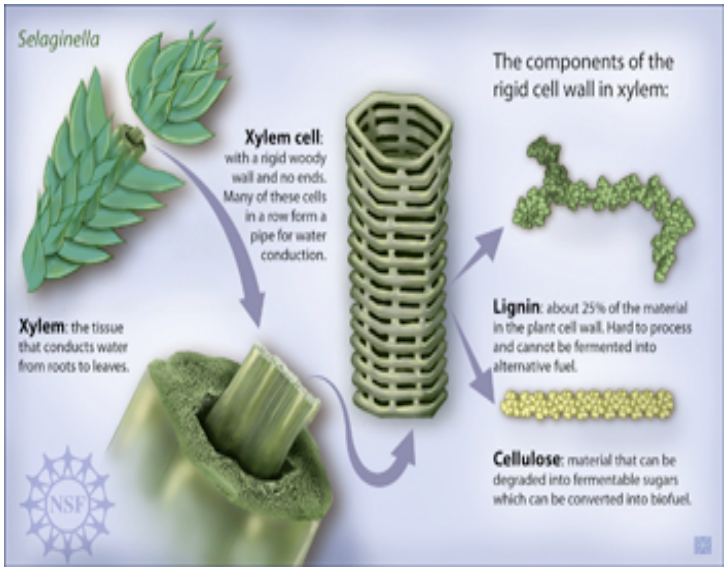
and

Part B: Chemoautotrophic archaea in the mesopelagic ocean

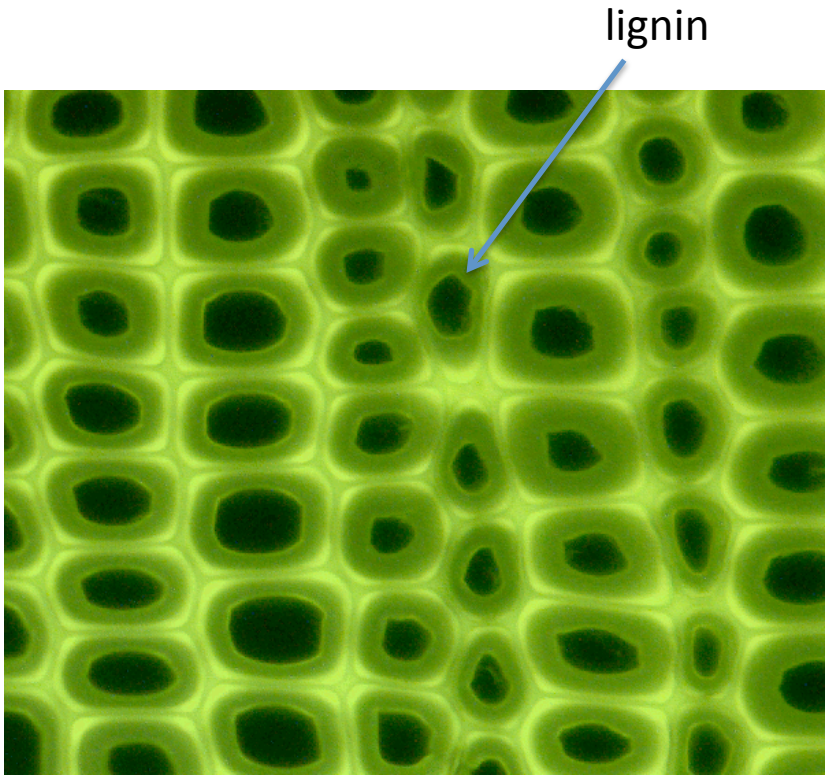
by Ann Person, TEXEL MOG group, John Hayes, and many, many others…..

## Is terrestrial organic carbon part of the mix of organic matter in marine sediments?

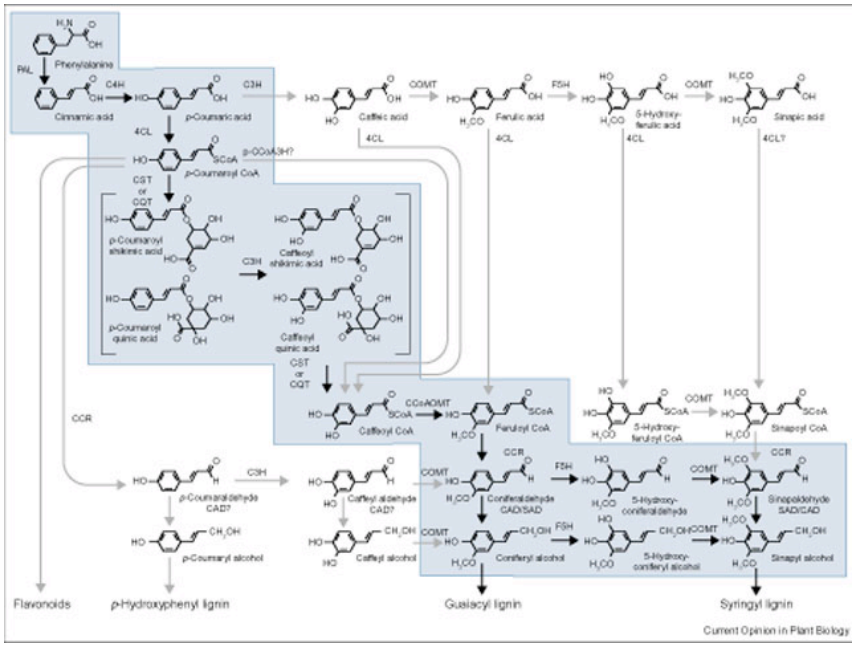
Lignin is a structural biopolymer that is an intimate part of cell walls in higher plants. It represents between 25-30% of the dry mass of wood. It also occurs in non-woody tissues. Liginin is hydrophilic, and therefore important in water transport processes with plants.




www.nsf.gov



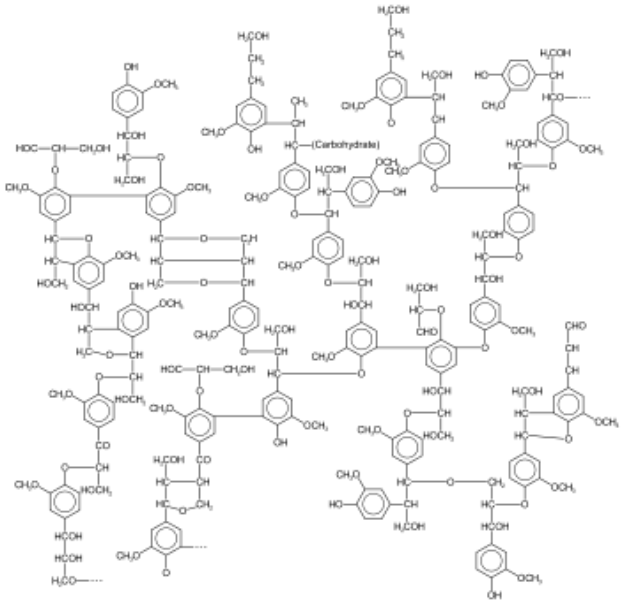

Fluorescence micrograph of wood cells. The lignin appears brighter due to fluorescence of aromatic functional groups. Lignin is a structural component that occurs between cells but also within cell walls. www.cb.uu.se/annual_report



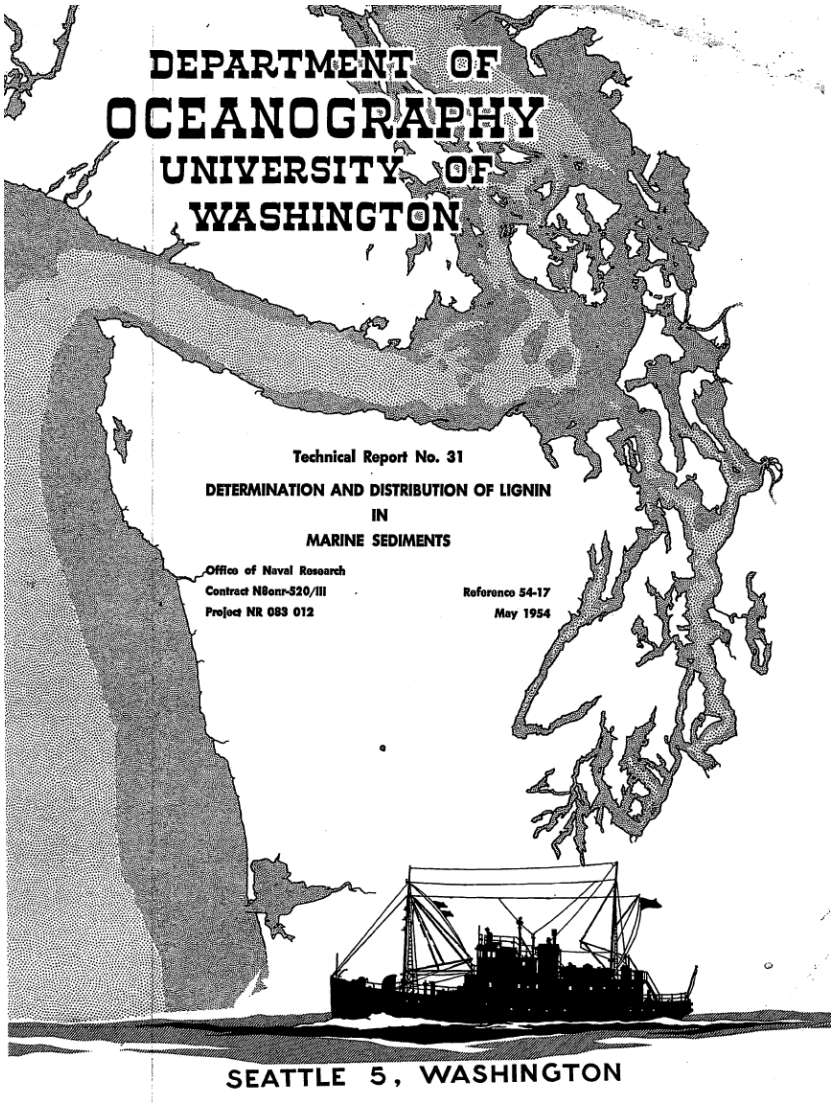

cellwall.genomics.purdue.edu

Biosynthesis of lignin: Synthesis occurs in the cytosol with the amino acid phenyl alanine. PA is deaminated, and the product (cinnamic acid) is hydroxylated and methylated. The different monolignols are attached to glucose, transported through the cell membrane to the apoplast, the glucose is removed and the monolinols are polymerized into lignin

Lignin is a complex biopolymer with a structure that is not completely understood. The monomers are lignols with different numbers of methoxy and hydroxy functional groups. Lignin monmers are linked together with ether bonds that are resistant to hydrolytic enzymes under a wide range of pH conditions. Bonds are much stronger than ester bonds found in common lipids or glycosidic linkages found in polysaccharides. This imparts a high degree of Resistance to biological degradation and chemical analysis. Lignins are degraded by peroxidases and perhaps free radicals Released by fungi in soils.



www.wikipedia.com




Lignin has a long history of analysis in marine sediments as a tracer for higher plant carbon, first reported by Trask (1932) And Waksman (1933). Early analysis relied on the resistance of lignin to most chemical extractions and acid catalyzed hydrolytic reactions to remove other fractions of organic carbon. Carbon that remained in sediments after aggressive solvent extraction and acid hydrolysis was assumed to be lignin. Many of the techniques were borrowed from wood and paper chemistry.

Lignin in Puget Sound sediments
From Bader (1954)

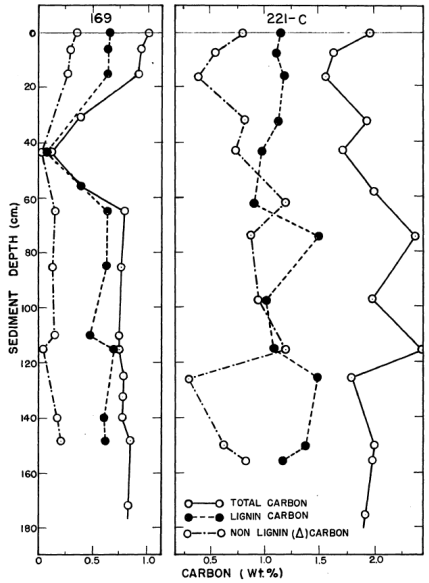



FIGURE 1. Vertical distribution of total organic carbon, "lignin"-carbon and non-lignin carbon in two cores of Puget Sound Sediments.

# Chemical analysis of lignin oxidation products in marine sediments

Mix sediments with $NaOH_{(aq)}$ and Cu(II)O

↓ 180°C, 2-4 hr

Δ pH = 1; liquid/liquid extraction into ether

↓

Dry, methylate OH to Trimethyl silyl (TMS) ethers

↓

Analysis by gas chromatography-mass spectrometry

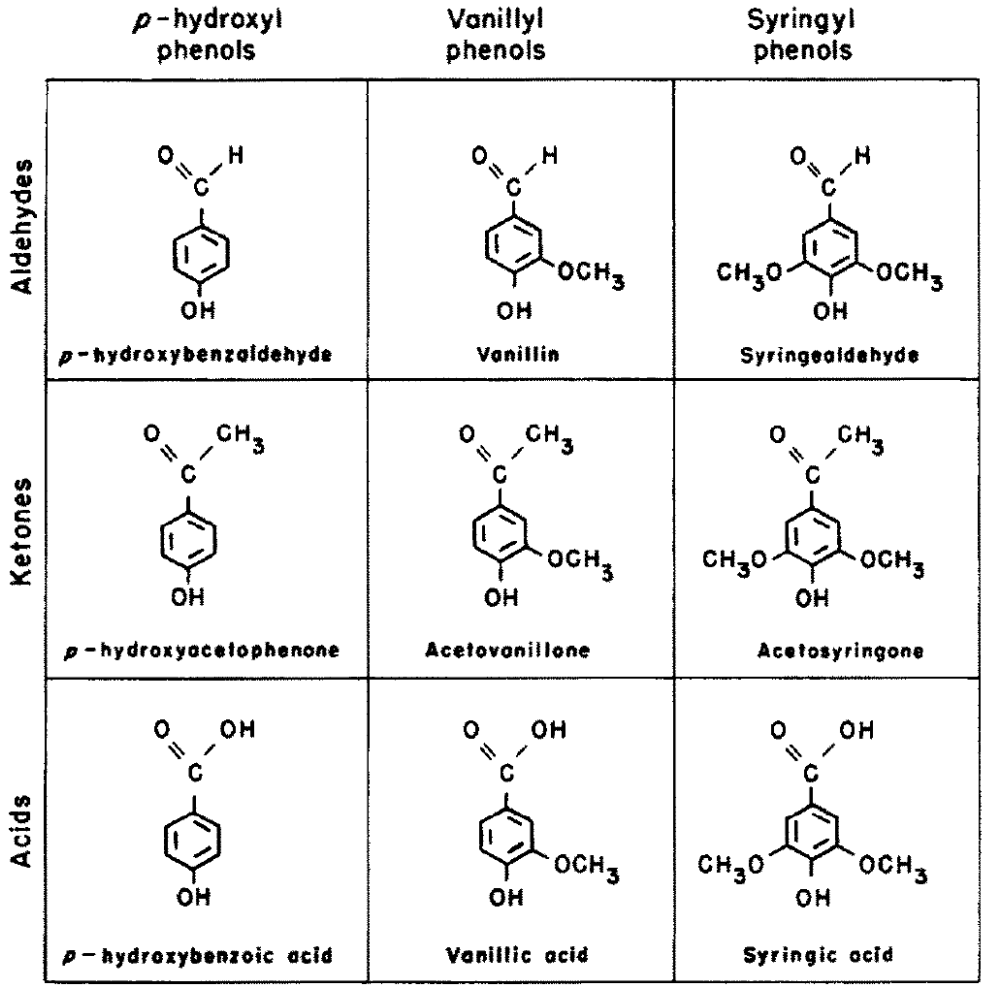



Fig. 2. Chemical structures of the nine index phenols.

Ertel and Hedges, Analy. Chem 1982 v 52 p 174

Hedges and Parker GCA v40, 1976 p 1019-1029

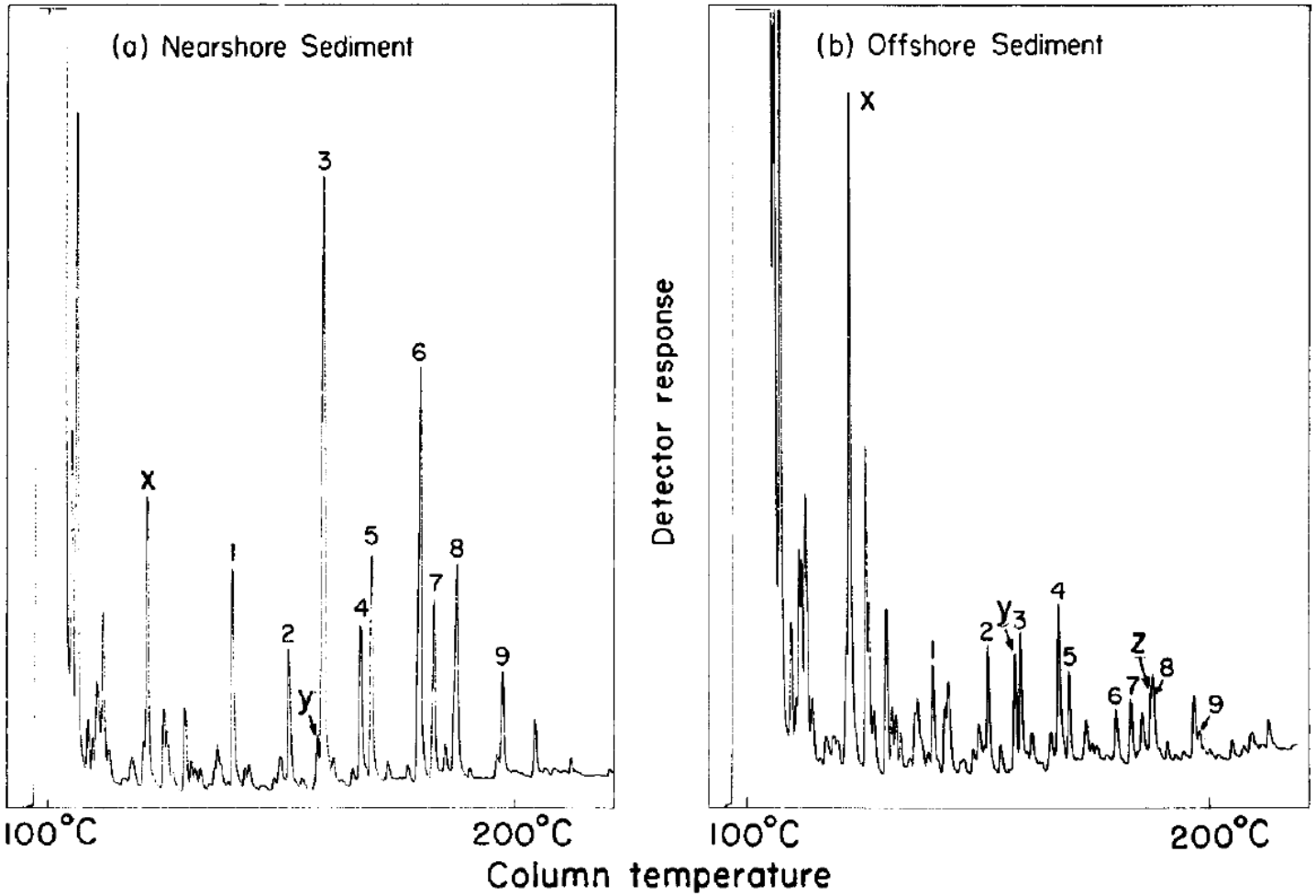



Fig. 3. Gas chromatograms of the oxidation product mixtures from typical nearshore and offshore sediments. The representative nearshore sediment (a) is Trinity River sample no. 1. The offshore sediment (b) is Trinity River sample no. 3. All compounds were converted to their trimethylsilyl derivatives. A 100-ft by 0.010-in. i.d. stainless steel column coated with Dexsil 300–GC liquid phase was temperature-programmed from 100 to 270°C at a rate of 4°/min. Peak identities: 1, *p*-hydroxybenzaldehyde; 2, *p*-hydroxyacetophenone; 3, vanillin; 4, *p*-hydroxybenzoic acid; 5, acetovanillone; 6, syringealdehyde; 7, vanillic acid; 8, acetosyringone; 9, syringic acid; x, benzoic acid; y, *m*-hydroxybenzoic acid; z, 3,5-dihydroxybenzoic acid.

Hedges and Parker GCA v40, 1976 p 1019-1029

# The distribution of LOPs are characteristic of different higher plant tissues

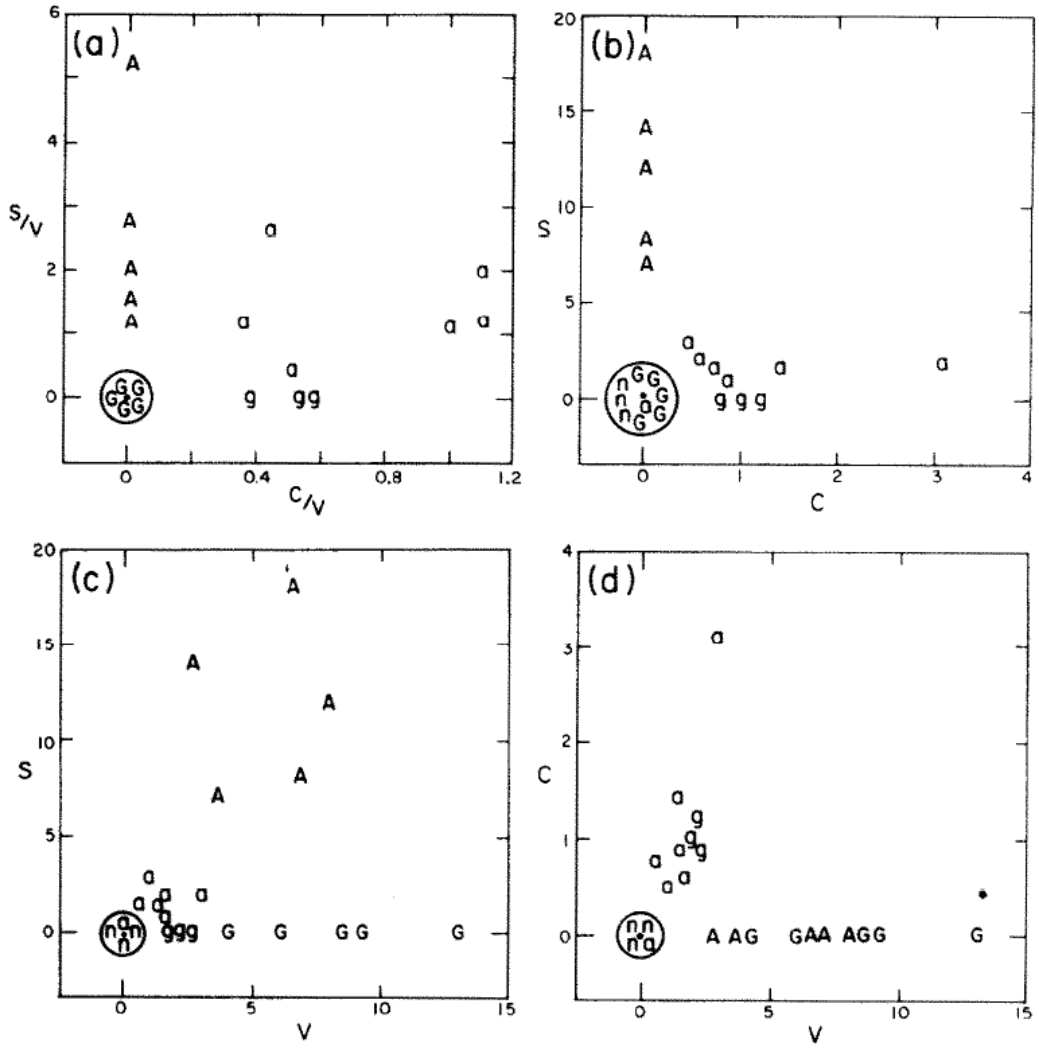



Fig. 1. Plots of lignin parameters for individual plant tissues in two dimensional compositional regions. Symbols: (G)—gymnosperm woods, (g)—nonwoody gymnosperm tissues, (A)—angiosperm woods, (a)—nonwoody angiosperm tissues, (n)—nonvascular plant tissues. All circled letters plot at the origin of the corresponding region

Hedges and Mann 1979 GCA v 43 p 1803-1807

# Lignin geochemistry in marine sediments from the coast of Washington

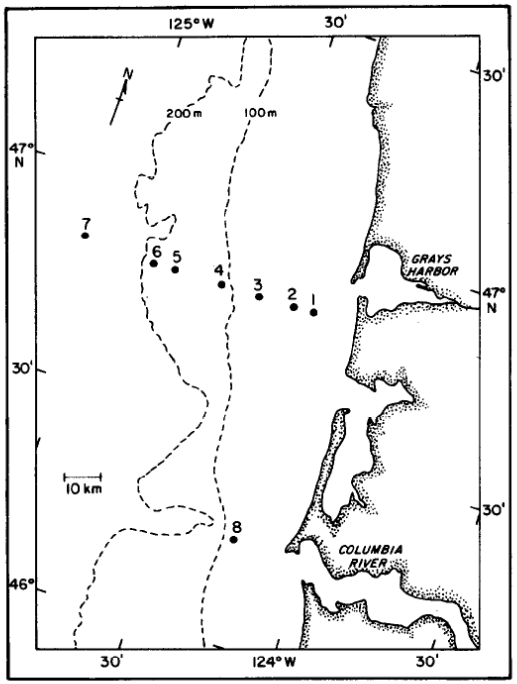
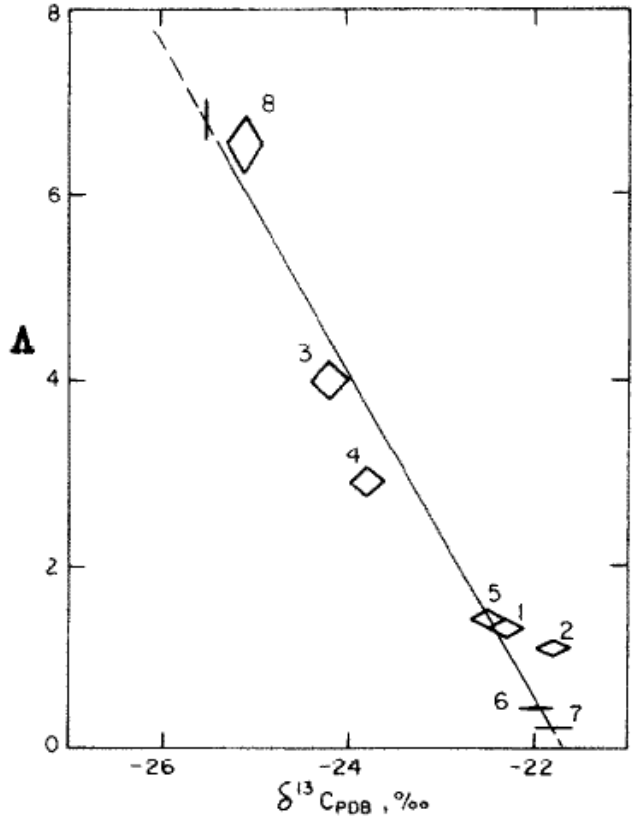



Fig. 1. Study area off the southern coast of Washington state with sediment core collection sites indicated by solid circles.

Terrestrial organic matter and sediment is delivered primarily by the Columbia River and drifts North/Northwest due to bottom currents. Inshore (0-60 m) are sands grading to finer grain sizes. At depths > 60m, sediments are silts. Seaward of silt deposits are relic beach sands.

Lignin oxidation products and $\delta\ ^{13}C$ are both tracers for terrestrial organic matter in marine sediments. LOPs provide a molecular tracer to compliment the use of stable isotopes as a bulk carbon tracers. Both have their own strengths and weaknesses as proxies. A regression of $\lambda$, the product of LOPs x the ratio of total terrestrial carbon to LOPs, and $\delta^{13}C$ yields a linear fit as expected between the two proxies- e.g. they both appear to be measuring the related properties

$\Lambda$ = $\Sigma$ vanillyl + syringyl + cinnamyl phenols / 100 mg carbon




Hedges and Mann 1979 GCA v 43 p 1803-1807

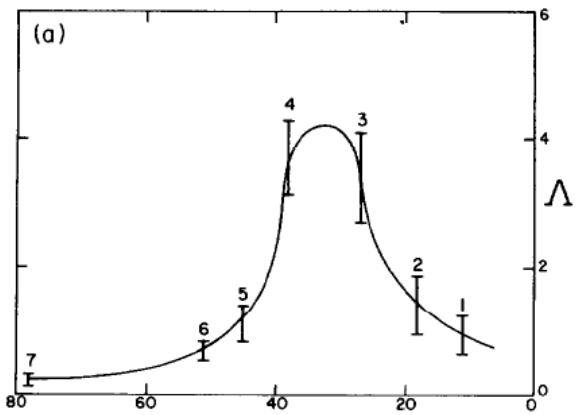



Fig. 2a. Average Λ values of sediment cores 1–7 plotted against distance offshore. Ranges correspond ± one standard deviation from the mean.

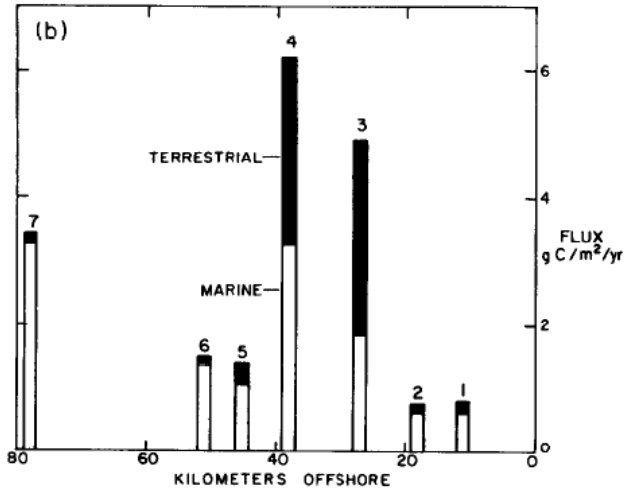



Fig. 2b. Marine and terrestrial components of the total effective flux of organic carbon into the uppermost 5 cm of sediment cores 1–7.

Pb-210 dating of sediments and seismic reflection data indicate there is a band of rapidly accumulating sediments on the mid shelf (35 km offshore). This is reflected in the very high values of Λ at this location, suggesting deposition of terrestrial plant material at this site.

There is a positive correlation between sediment accumulation rate and L, suggesting terrestrial organic and mineral materials are deposited together, although not necessarily as the same phase. Yields of LOP normalized to total carbon are similar to values obtained on plant fragments.

The % marine and % errestrial are deduced from the Isotope mixing model of $\delta^{13}C$ vs Λ.

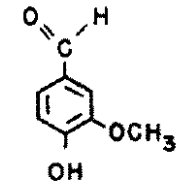
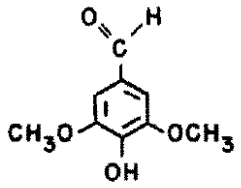
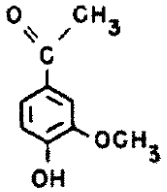
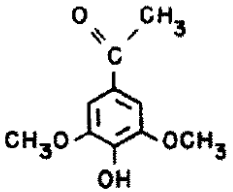
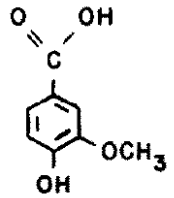
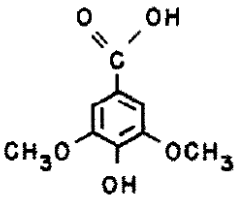
Hedges and Mann 1979 GCA v 43 p 1803-1807

Table 2. Percentages of organic carbon and lignin parameters for individual sediment core horizons along with whole core averages

| Core water depth distance offshore | Depth interval (cm) | Organic carbon (wt%) | Λ | S/V | C/V |
|---|---|---|---|---|---|
| 5 | 0–5 | 1.05 | 1.4 | 0.29 | 0.12 |
| 145 m | 5–10 | 0.97 | 0.88 | 0.24 | 0.13 |
| 45 km | 10–15 | 0.81 | 1.3 | 0.25 | 0.10 |
| | 15–20 | 0.80 | 0.80 | 0.28 | 0.15 |
| | 20–25 | 0.76 | 0.90 | 0.31 | 0.16 |
| | means | 0.89 ± 0.12 | 1.1 ± 0.3 | 0.27 ± 0.03 | 0.13 ± 0.02 |
| 6 | 0–5 | 0.58 | 0.45 | 0.17 | 0.08 |
| 175 m | 5–10 | 0.58 | 0.68 | 0.20 | 0.11 |
| 51 km | 10–15 | 0.54 | 0.75 | 0.25 | 0.10 |
| | 15–20 | 0.61 | 0.61 | 0.20 | 0.13 |
| | 20–25 | 0.74 | 0.85 | 0.40 | 0.07 |
| | means | 0.61 ± 0.08 | 0.67 ± 0.15 | 0.24 ± 0.09 | 0.10 ± 0.02 |
| 7 | 0–5 | 2.58 | 0.23 | 0.31 | 0.49 |
| 620 m | 5–10 | 2.55 | 0.19 | 0.29 | 0.57 |
| 78 km | 10–15 | 2.62 | 0.26 | 0.32 | 0.31 |
| | 15–20 | 2.47 | 0.22 | 0.26 | 0.30 |
| | 20–25 | 2.25 | 0.31 | 0.38 | 0.37 |
| | 25–30 | 2.06 | 0.19 | 0.30 | 0.26 |
| | 30–35 | 1.67 | 0.22 | 0.25 | 0.24 |
| | 35–40 | 1.36 | 0.19 | 0.43 | 0.47 |
| | means | 2.20 ± 0.47 | 0.23 ± 0.04 | 0.32 ± 0.06 | 0.38 ± 0.12 |

Intervals about mean values are ±1 standard deviation.

Within each class of LOP, there is a difference in the degree of Oxidation, which may be useful to look at degradation pathways

| Vanillyl phenols | Syringyl phenols |
|---|---|
|  Vanillin |  Syringealdehyde |
|  Acetovanillone |  Acetosyringone |
|  Vanillic acid |  Syringic acid |

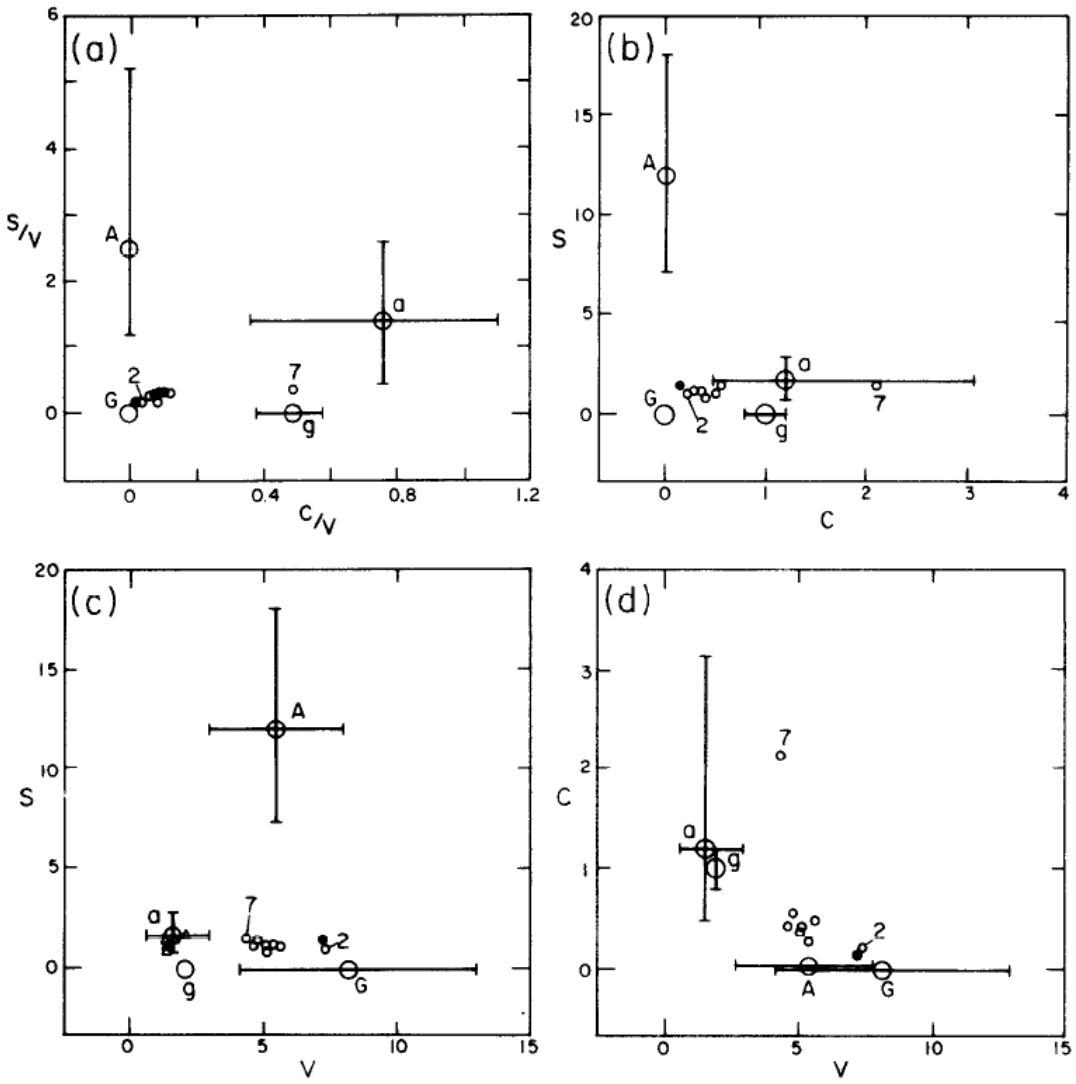



Fig. 5. Lignin parameter plots for surface sediments from the Washington coast (open circles), coarse plant debris collected near the mouth of the Columbia River (solid circle), and surface sediments from near the mouth of the Mississippi River (open triangles in section c). Also shown are lignin parameter ranges around mean values for the following categories of plant materials: gymnosperm woods (G), nonwoody gymnosperm tissues (g), angiosperm woods (A), and nonwoody angiosperm tissues (a); (HEDGES and MANN, 1979).

# Oxidation of Lignin phenols as a measure of lignin degradation

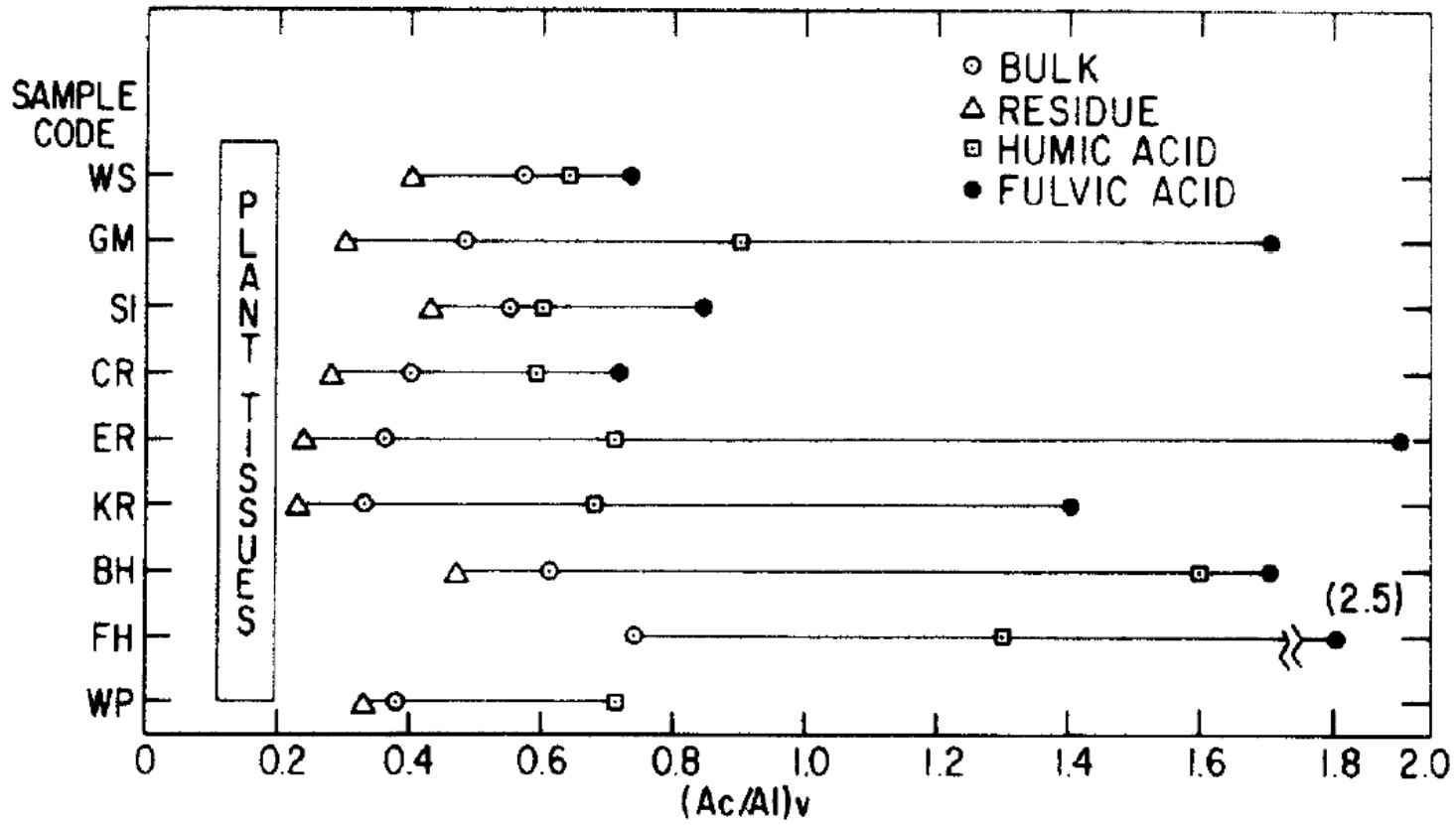
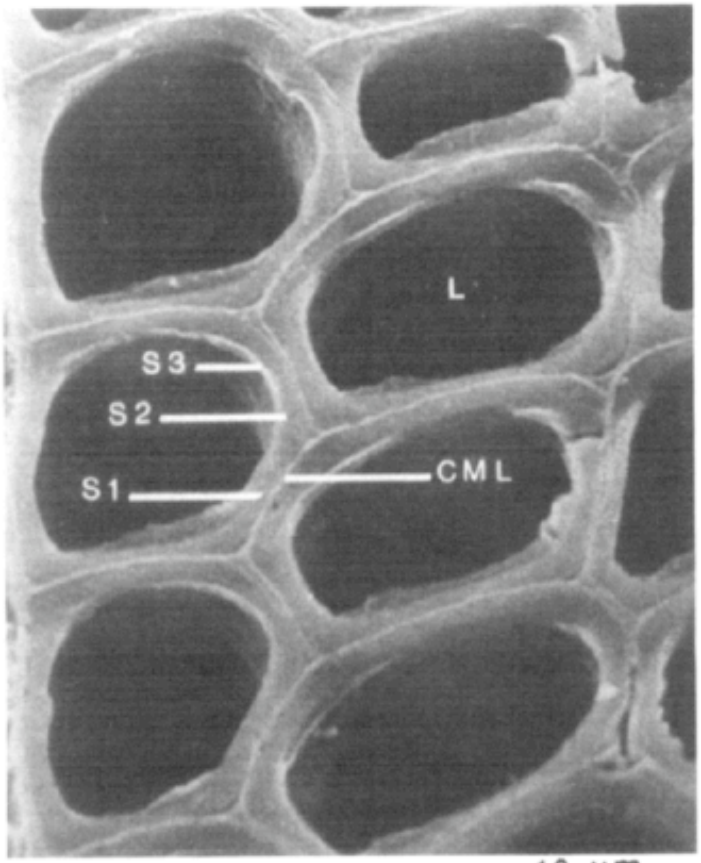



FIG. 4. Weight ratios of vanillic acid to vanillin, (Ac/Al)v, for the bulk samples and the humic, fulvic and base-insoluble residual fractions. The range indicated for plant tissues (0.15 ±0.05) is calculated from 19 plant tissues (HEDGES and MANN, 1979a). Sample codes are in Table 1.

Ertel and Hedges GCA (1984) v 48 p 2865

# Elemental and compositional changes in vascaular plant biopolymer composition with diagenesis

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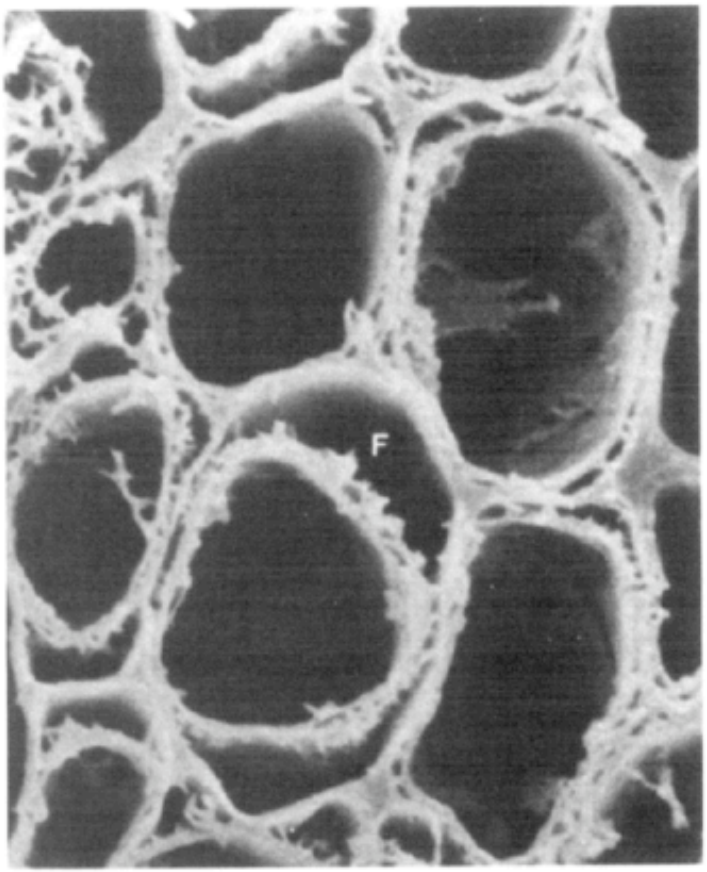



b

# Elemental and compositional changes in vascaular plant biopolymer composition with diagenesis

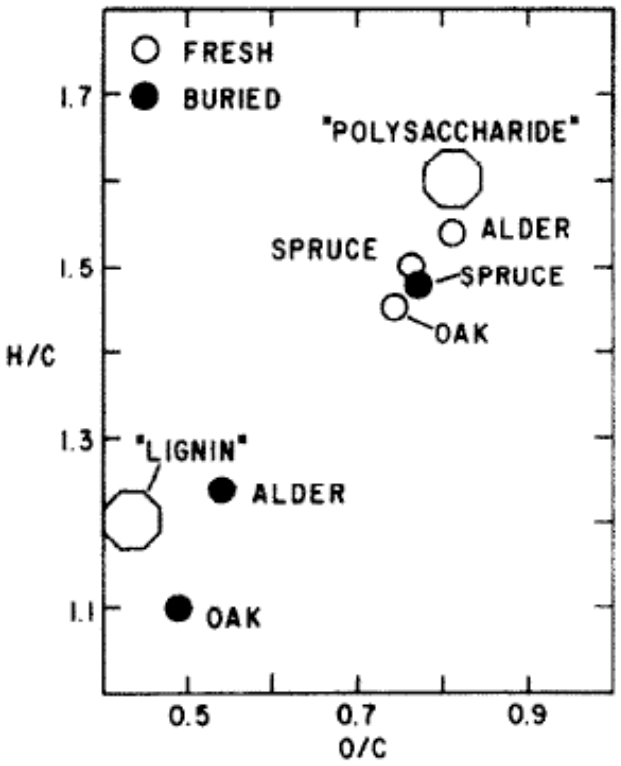



FIG. 2. Van Krevelen plot of modern and buried woods (Table 1). The "LIGNIN" composition is the average for 8 hardwood lignins taken from SARKANEN and LUDWIG (1971, p. 68). The "POLYSACCHARIDE" composition was calculated for an "average hardwood" assuming 45 wt% hexosan and 25 wt% glucuronoxylan (SJÖSTRÖM, 1981).

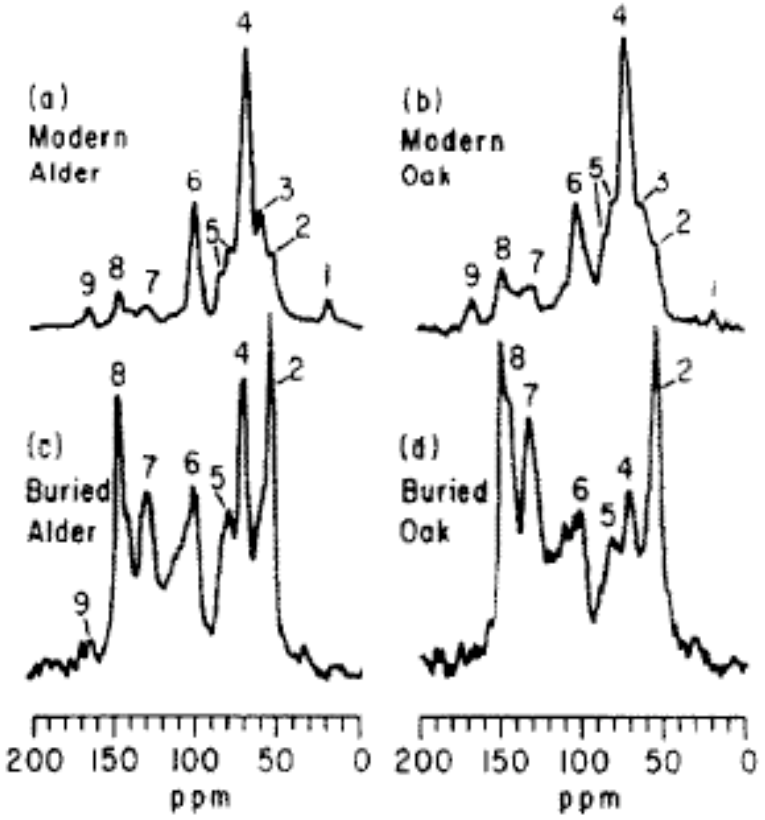



FIG. 3. CP-MSA $^{13}C$ NMR spectra of (a) modern alder, (b) modern oak, (c) buried alder, and (d) buried oak. Assignments for the numbered peaks are given in Table 2.

Table 4. Lignin phenol yields and compositional parameters for modern and buried woods[a]

| Sample (parameter) | ΣP | ΣV | ΣS | Σ9[b] | A[c] | P/V[d] | S/V[d] | (Ad/Al)v[e] | (Ad/Al)s[e] |
|---|---|---|---|---|---|---|---|---|---|
| Modern spruce | 0.05 | 5.05 | 0 | 5.10 | 10.9 | 0.01 | 0 | 0.13 | -- |
| Buried spruce | 0.03 | 5.39 | 0 | 5.42 | 11.7 | 0.01 | 0 | 0.20 | -- |
| (% of modern) | 60 | 107 | - | 106 | | | | | |
| (% preserved) | 56 | 100** | - | 99 | | | | | |
| Modern alder | 0.06 | 2.65 | 6.86 | 9.57 | 21.1 | 0.02 | 2.58 | 0.13 | 0.14 |
| Buried alder | 0.11 | 6.16 | 11.1 | 17.4 | 32.1 | 0.02 | 1.79 | 0.14 | 0.14 |
| (% of modern) | 183 | 234 | 162 | 182 | | | | | |
| (% preserved) | 78 | 100** | 69 | 78 | | | | | |
| Modern oak* | 0.02 | 2.07 | 6.90 | 9.00 | 19.0 | 0.01 | 3.34 | 0.11 | 0.09 |
| Buried oak* | 0.08 | 6.41 | 10.0 | 16.5 | 28.9 | 0.01 | 1.56 | 0.21 | 0.24 |
| (% of modern) | 400 | 310 | 145 | 183 | | | | | |
| (% preserved) | 120 | 100** | 47 | 59 | | | | | |

[a] Yields are mg/100mg of ash-free sample unless otherwise indicated.
[b] Σ9 = ΣP + ΣV + ΣS.
[c] Total syringyl and vanillyl phenol yield (mg) normalized to 100 mg of organic carbon.
[d] Weight ratio of total *p*-hydroxyl or total syringyl phenols to total vanillyl phenols.
[e] Weight ratio of acidic to aldehydic phenol in the vanillyl and syringyl families.
* Sample done in duplicate (mean values given).
** No degradation is assumed (see text).

Abbreviations: ΣP, sum of *p*-hydroxyl phenols (*p*-hydroxybenzaldehyde + *p*-hydroxyacetophenone + *p*-hydroxybenzoic acid); ΣV, sum of vanillyl phenols (vanillin + acetovanillon + vanillic acid); ΣS, sum of syringyl phenols (syringaldehyde + acetosyringone + syringic acid).

# The determination of terrestrial organic matter in sediments *via* lignin oxidation products

Lignin is a structural polymer present in all vascular plants, but not present in marine microalgae. Serves as a proxy for vascular plants in marine sediments.

We do not analyze the in-tact polymer, but rather its oxidation products. Polymerization occurs by ether bonds, which are hard to break. This explains their resistance to degradation. Fungi use peroxidases to degrade them in soils.

In the lab, we use alkaline cupric oxidation at high temperature to depolymerize and oxidize ether linkages. Once this has been done, aqueous solutions are made acid, and LOP extracted into organic solvents. The LOP are dried and made into volatile trimethylsilyl ethers for gas chromatography-mass spectrometric analysis.

The distribution of lignin phenols (syringyl, vanillyl, and cinnamic; S:VC) in LOPs is very characteristic for specific types of higher plants (flowering vs non-flowering) and tissue types (woody vs non-woody). These distributions can often be used to characterize the type of vascular plant material in a sample.

The amount of lignin is marine samples is a proxy for the amount of terrestrial organic matter, and has been shown to co-vary with $\delta^{13}C$ and other higher plant lipid biomarkers.

The oxidation state of LOP can be used to assess the relative degree of degradation in lignin between samples, although this is not an absolute proxy- care must be taken when applying this tool.

In-tact polar (ether) lipids as proxies for archaeal distribution and metabolism in the mesopelagic ocean

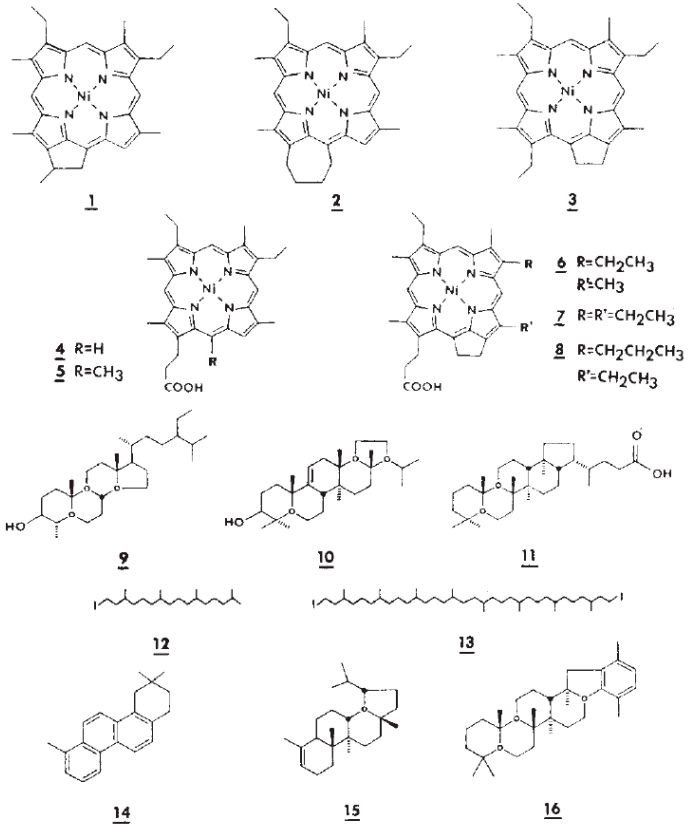



**Fig. 1** Structures of compounds for which isotopic compositions are reported. For methods of isolation and analysis see references cited in Table 2.

**Table 1** Isotopic compositions of carbon fractions in the Messel shale

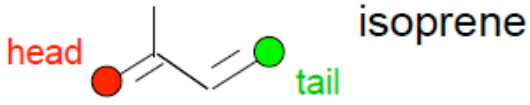
| Sample identification | $\delta^{13}C_{PDB}$ (‰) |
|---|---|
| Total carbonate | +7.34 ± 0.12 |
| Total organic carbon, kerogen* | −28.21 ± 0.03 |
| Total extractable organic material† | −29.72 ± 0.10 |
| Total extract fractionated on $SiO_2$ column:‡ | |
| Hexane eluent | −33.66 ± 0.14 |
| Toluene eluent | −29.66 ± 0.03 |
| Methanol eluent | −28.30 ± 0.07 |
| Alkyl porphyrin fraction (Strasbourg) | −22.60 ± 0.08 |
| Total porphyrins (Bloomington) | −23.43 ± 0.06 |
| Acid porphyrin fraction (Strasbourg) | −23.91 ± 0.04 |

**Table 2** Carbon isotopic compositions of individual biomarkers

| Structure* | Molecular precursor† | Refs‡ | Related organism§ | Abundance ng $g^{-1}$ | $\delta^{13}C_{PDB}$(‰) | $n^{\|}$ |
|---|---|---|---|---|---|---|
| 2 | | 6 | | 1 | −19.50 ± 0.05 | 3 |
| 5 | | 7 | *Algae* | 2 | −21.58 ± 0.13 | 1 |
| 4 | | 7 | *Algae* | 1 | −21.89 ± 0.15 | 2 |
| 3 | | 6 | *Algae* | 4 | −21.92 ± 0.08 | 2 |
| 1 | Chl c | 6 | Algae | 5 | −22.15 ± 0.03 | 2 |
| 6 | | 8 | *Mixture* | 12 | −23.12 ± 0.04 | 3 |
| 8 | Bchl d | 8 | Photosynth. bacteria | 3 | −23.92 ± 0.05 | 3 |
| 7 | Bchl d | 8 | Photosynth. bacteria | 1 | −23.96 ± 0.06 | 2 |
| 9 | | 11 | Dinoflagellate | | −25.37 ± 0.08 | 1 |
| 10 | | 18 | Unknown | | −28.05 ± 0.03 | 1 |
| 12 | Phytanyl ether | ¶ | Methanogen | ~8 | −29.74 ± 0.14 | 3 |
| 13 | Biphytanyl ether | ¶ | Methanogen | ~16 | −29.88 ± 0.12 | 3 |
| 11 | | 5 | Bacteria | | −32.27 ± 0.11 | 2 |

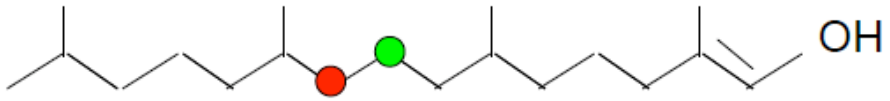
Hayes et al. Nature 1987

# Isoprenoid lipids - Configurations




*Regular isoprenoids*

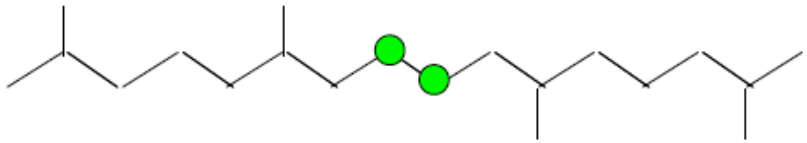
- head-to-tail
  - e.g. phytol



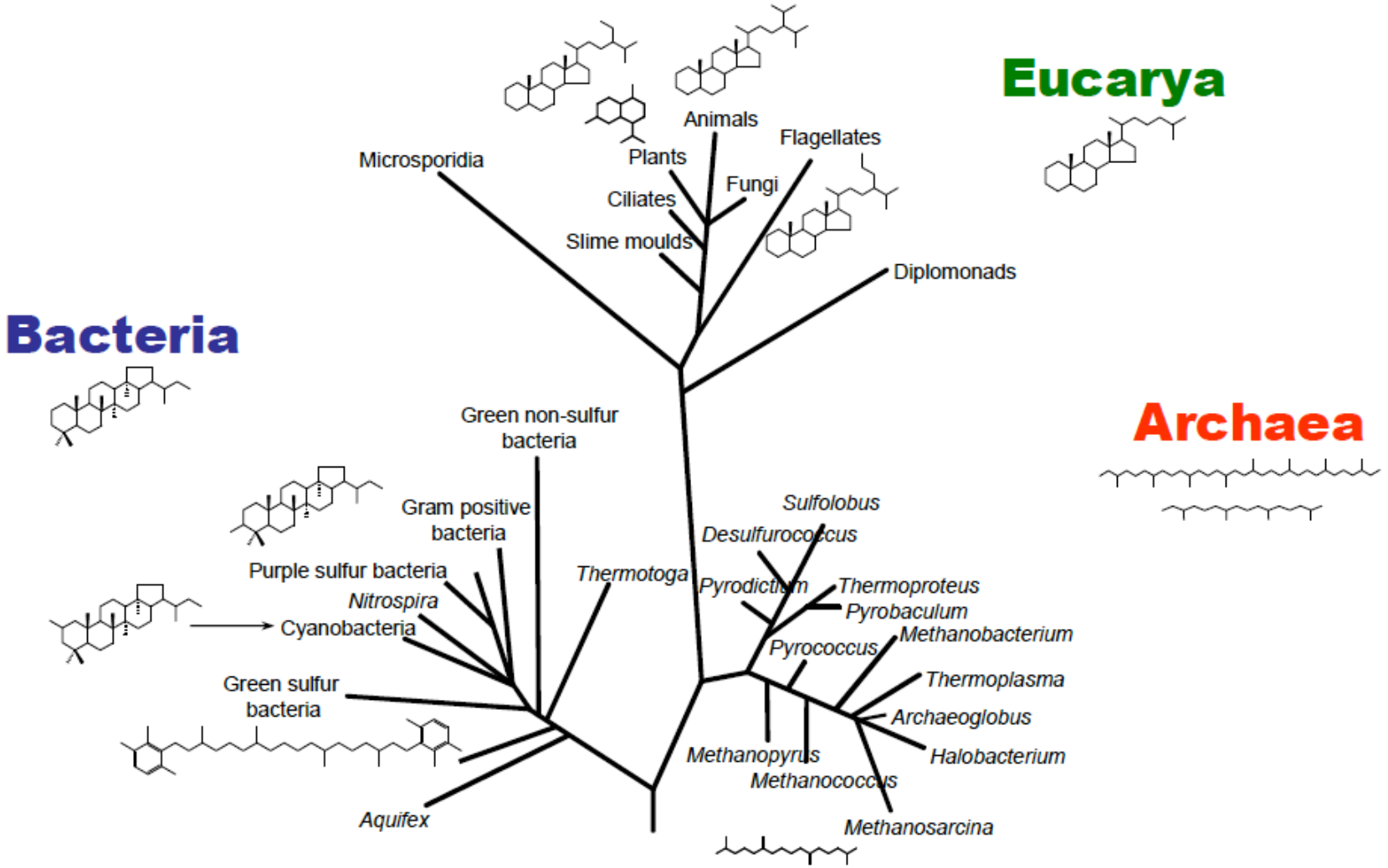

*Irregular isoprenoids*

- head-to-head

- tail-to-tail

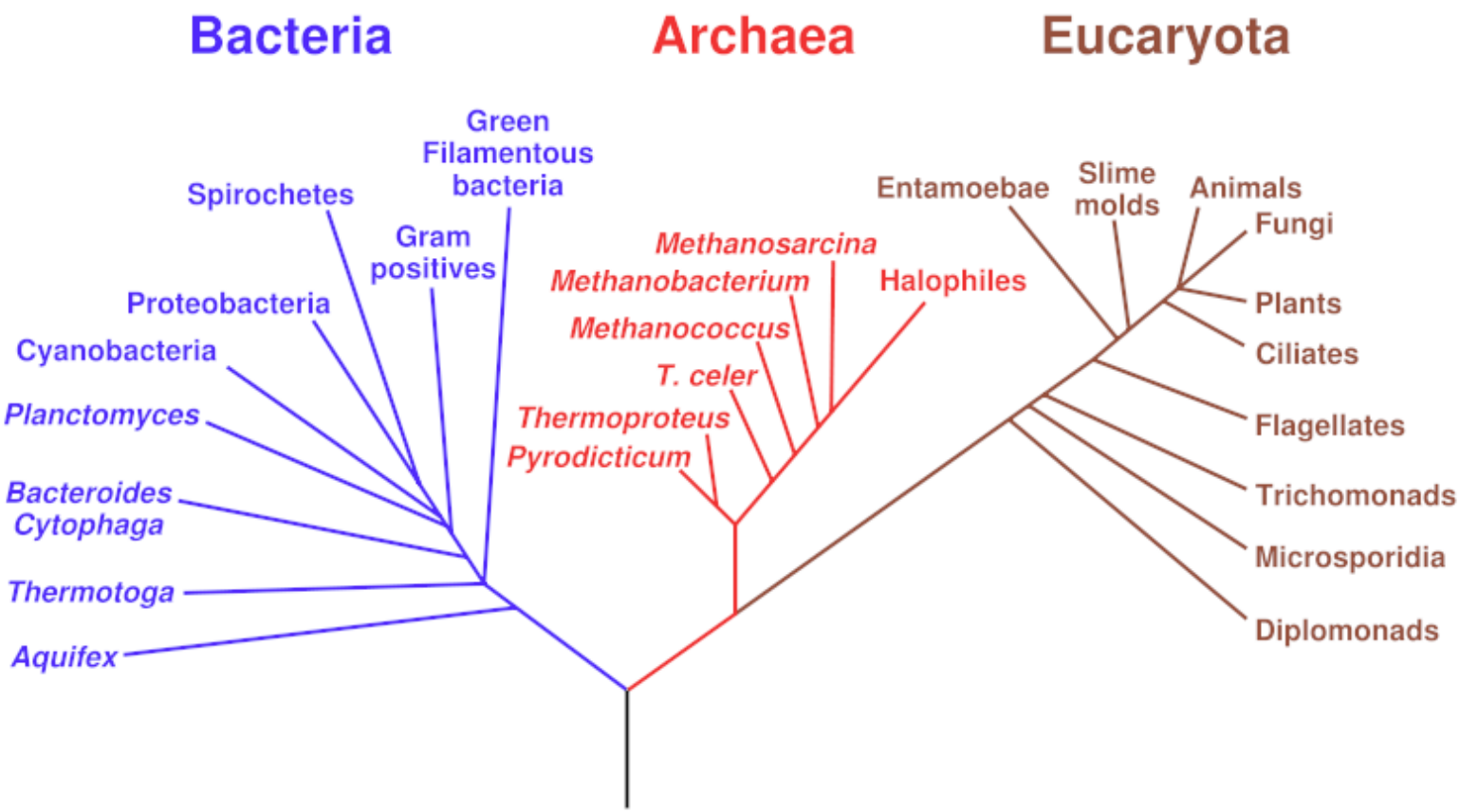


# …and their Molecular Fossils




*rRNA tree modified after Woese*

# Phylogenetic Tree of Life




Slide courtesy of Ann Pearson, Harvard University

*Proc. Natl. Acad. Sci. USA*
Vol. 89, pp. 5685–5689, June 1992
Ecology

# Archaea in coastal marine environments

(archaebacteria/phylogeny/bacterioplankton/molecular ecology)

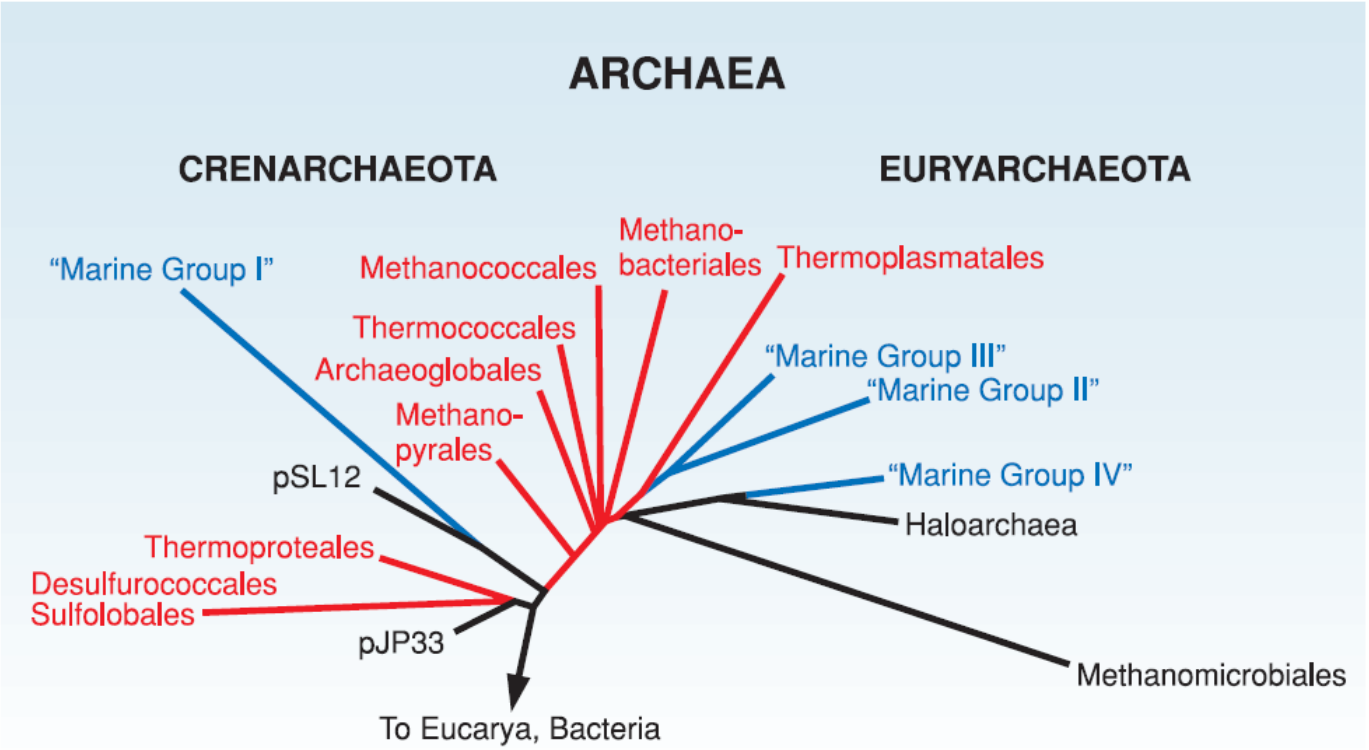
EDWARD F. DELONG*

- Archaea had been considered "extremophiles".
- Temperate, aerobic Archaea were unexpected.

**ABSTRACT** Archaea (archaebacteria) are a phenotypically diverse group of microorganisms that share a common evolutionary history. There are four general phenotypic groups of archaea: the methanogens, the extreme halophiles, the sulfate-reducing archaea, and the extreme thermophiles. In the marine environment, archaeal habitats are generally limited to shallow or deep-sea anaerobic sediments (free-living and endosymbiotic methanogens), hot springs or deep-sea hydrothermal vents (methanogens, sulfate reducers, and extreme thermophiles), and highly saline land-locked seas (halophiles). This report provides evidence for the widespread occurrence of unusual archaea in oxygenated coastal surface waters of North America. Quantitative estimates indicated that up to 2% of the total ribosomal RNA extracted from coastal bacterioplankton assemblages was archaeal. Archaeal small-subunit ribosomal RNA-encoding DNAs (rDNAs) were cloned from mixed bacterioplankton populations collected at geographically distant sampling sites. Phylogenetic and nucleotide signature analyses of these cloned rDNAs revealed the presence of two lineages of archaea, each sharing the diagnostic signatures and structural features previously established for the domain Archaea. Both of these lineages were found in bacterioplankton populations collected off the east and west coasts of North America. The abundance and distribution of these archaea in oxic coastal surface waters suggests that these microorganisms represent undescribed physiological types of archaea, which reside and compete with aerobic, mesophilic eubacteria in marine coastal environments.

Slide courtesy of Ann Pearson, Harvard University

FIGURE 1




Schematic tree showing the relationships of the four major groups of planktonic marine *Archaea* relative to cultivated groups.

DeLong, 2003

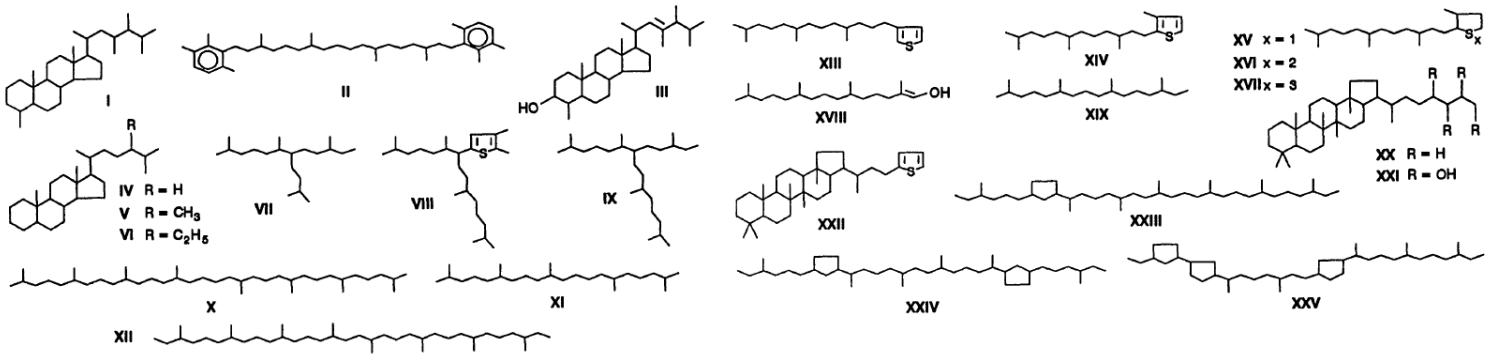
Slide courtesy of Ann Pearson, Harvard University

# Recognition of Paleobiochemicals by a Combined Molecular Sulfur and Isotope Geochemical Approach

Math E. L. Kohnen,* Stefan Schouten, Jaap S. Sinninghe Damsté,†
Jan W. de Leeuw, Dawn A. Merritt, J. M. Hayes

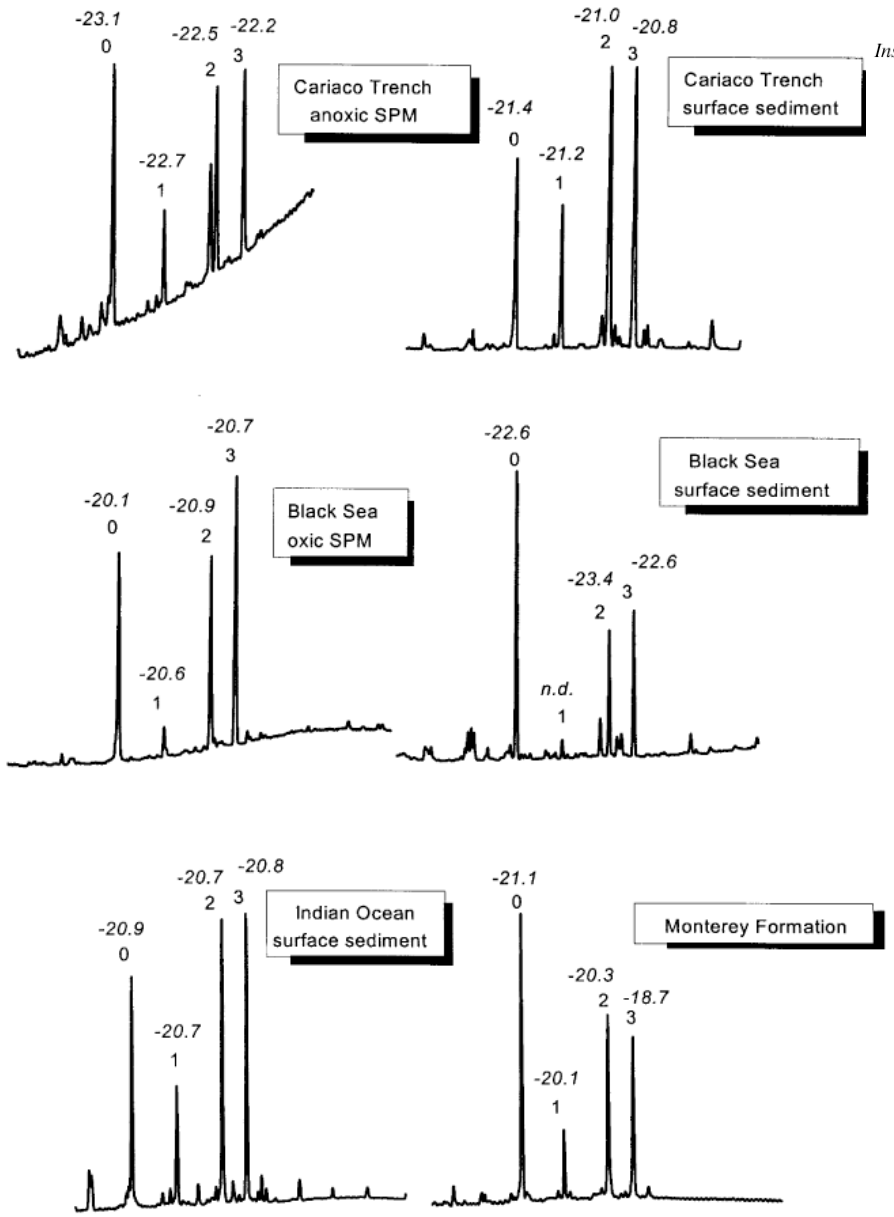
Study of organic matter in immature sediments from a Messinian evaporitic basin shows that consideration of structures, modes of occurrence, and carbon isotopic compositions of free and sulfur-bound carbon skeletons allow identification of biochemical precursors. Detailed information concerning biotic communities present during deposition of sediments can be retrieved in this way. Moreover, unprecedented biochemicals were recognized; these extend the horizon of biomarker geochemistry.

SCIENCE • VOL. 256 • 17 APRIL 1992




| | | | $\delta^{13}C$ (‰) | | |
|---|---|---|---|---|---|
| **XII** | E-bd | ND | −23.9 | gly di- or tetraether | Meth. archbact. |
| **XXIII** | E-bd | ND | −21.1 | gly tetraether | Meth. archbact. |
| **XXIV** | E-bd | ND | −20.5 | gly tetraether | Meth. archbact. |
| **XXV** | E-bd | ND | −20.6 | gly tetraether | Meth. archbact. |

Slide courtesy of Ann Pearson, Harvard University




Ether Lipids of Planktonic Archaea in the Marine Water Column†

MARCEL J. L. HOEFS,[1] STEFAN SCHOUTEN,[2]* JAN W. DE LEEUW,[1,2] LINDA L. KING,[3] STUART G. WAKEHAM,[3] AND JAAP S. SINNINGHE DAMSTÉ[1,2]

*Institute of Earth Sciences, Utrecht University, 3508 TA Utrecht,[1] and Division of Marine Biogeochemistry and Toxicology, Netherlands Institute for Sea Research, 1797 SZ 't Horatje, Texel,[2] The Netherlands, and Skidaway Institute of Oceanography, Savannah, Georgia 31411[3]*

Received 4 March 1997/Accepted 4 June 1997

Average $\delta^{13}C$ = -21 ± 1 ‰

Agrees with Kohnen paper

***"Compound-specific isotope analyses of the carbon skeletons suggest that planktonic Archaea utilize an isotopically heavy carbon source such as algal carbohydrates and proteins or dissolved bicarbonate."***

Slide courtesy of Ann Pearson, Harvard University

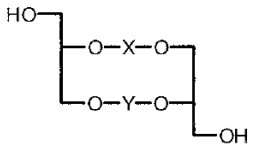
# Widespread occurrence of structurally diverse tetraether membrane lipids: Evidence for the ubiquitous presence of low-temperature relatives of hyperthermophiles

**Stefan Schouten*, Ellen C. Hopmans, Richard D. Pancost, and Jaap S. Sinninghe Damsté**

Netherlands Institute for Sea Research, Department of Marine Biogeochemistry and Toxicology, P.O. Box 59, 1790 AB Den Burg, The Netherlands

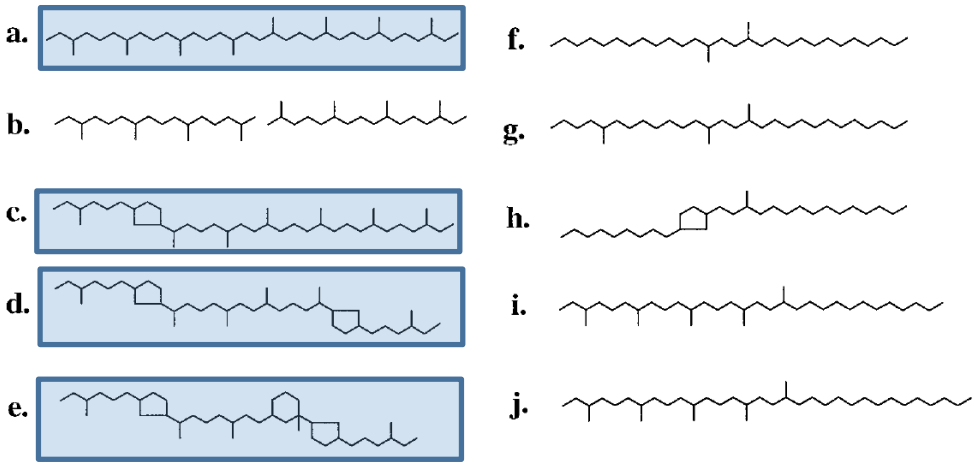
PNAS | **December 19, 2000** | vol. 97 | no. 26 | **14421–14426**

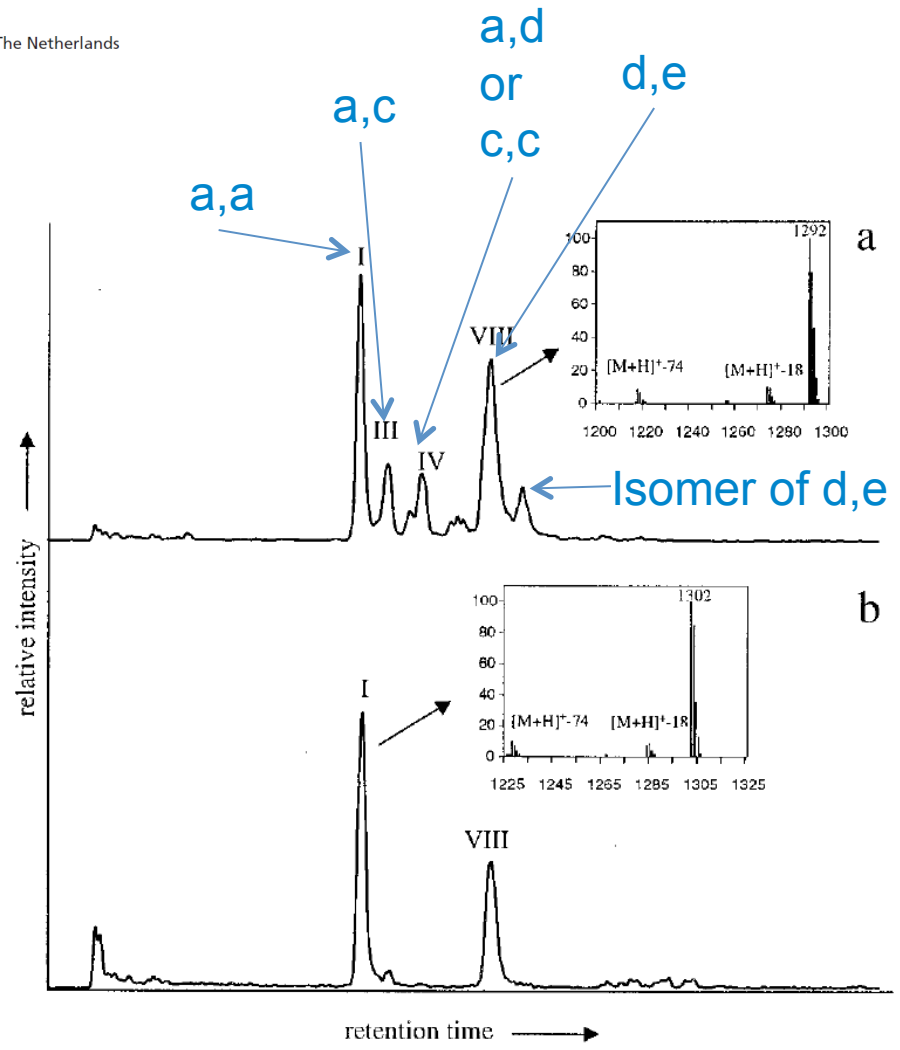
**Only the blue side-chains a, b, c, and d are archaeal**




GDGT basic structure

| GDGT no. | X | Y | GDGT no. | X | Y |
|---|---|---|---|---|---|
| I | a | a | IX | b | c |
| II | a | b | X | f | f |
| III | a | c | XI | f | g |
| IV | c | c | XII | g | g |
| V | c | d | XIII | f | h |
| VI | d | d | XIV | h | h |
| VII | a | d | XV | i | j |
| VIII | d | e | | | |



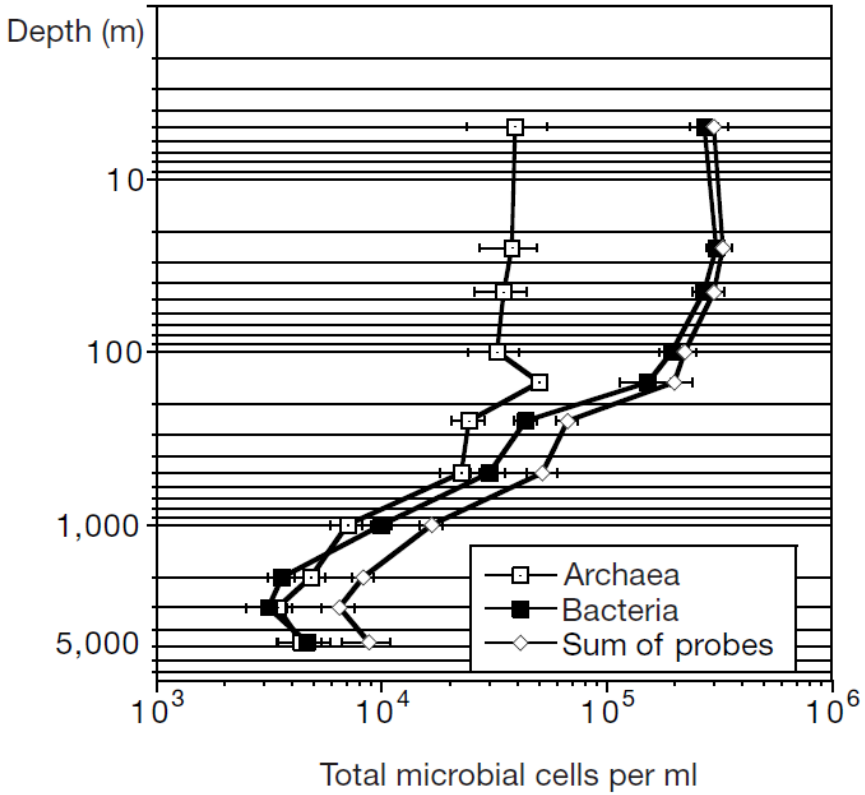



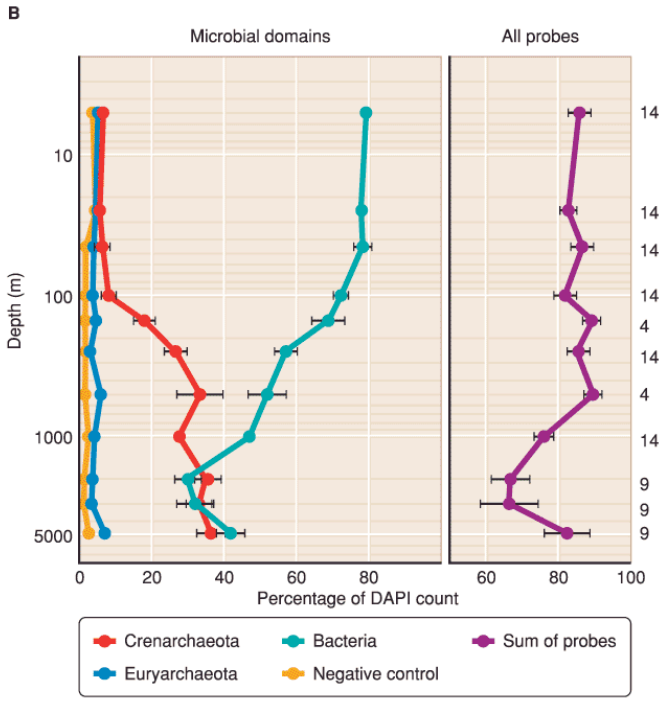

Slide courtesy of Ann Pearson, Harvard University

# Archaeal dominance in the mesopelagic zone of the Pacific Ocean

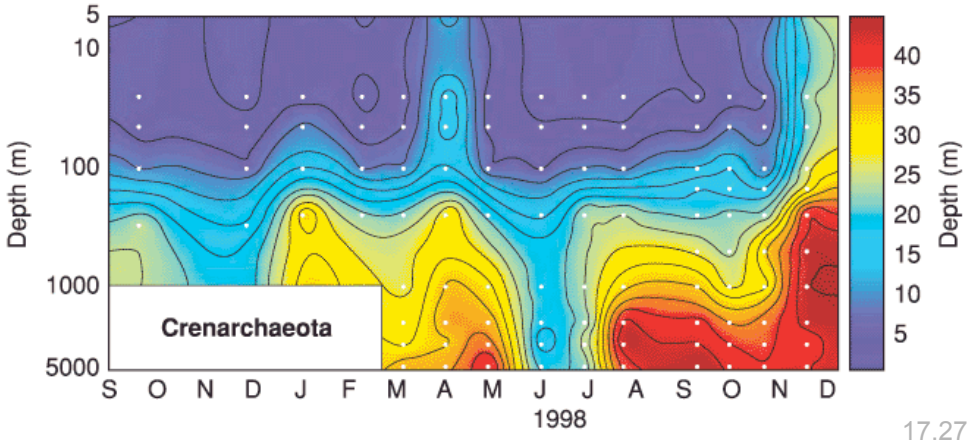
**Markus B. Karner*, Edward F. DeLong† & David M. Karl***

** University of Hawai'i, Department of Oceanography, 1000 Pope Road, Honolulu, Hawai'i 96822, USA*
*† Monterey Bay Aquarium Research Institute, 7700 Sandholdt Road, Moss Landing, California 95039, USA*




Abundance of planktonic *Archaea* at the Hawaii Ocean Time series station as a function of depth, determined by fluorescence in situ hybridization counts. Percentage of Marine Group I *Archaea* relative to total microbial counts in 2A is indicated by color; red, 40% of cell total; dark blue, 0% of cell total. Figure 2B shows the distribution of cell types determined by FISH, averaged over one year. *Crenarchaeota* refers specifically to Marine Group I *Archaea,* and *Euryarchaeota* refers specifically to Marine Group II *Archaea.* From Karner, M., E. F. DeLong, and D. M. Karl. 2001. Archaeal dominance in the mesopelagic zone of the Pacific Ocean. Nature **409:**507–510, with permission.




Slide courtesy of Ann Pearson, Harvard University

# Isolation of an autotrophic ammonia-oxidizing marine archaeon

Martin Könneke[1]*†, Anne E. Bernhard[1]*†, José R. de la Torre[1]*, Christopher B. Walker[1], John B. Waterbury[2] & David A. Stahl[1]

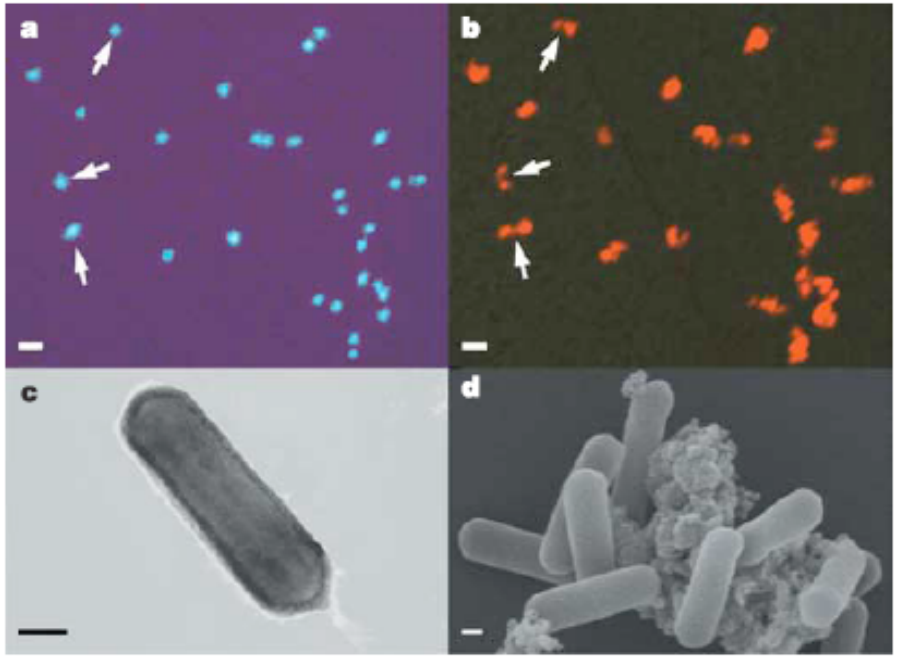



**Figure 2 | Photomicrographs of SCM1.** **a**, **b**, Fluorescence image of cells in identical fields of view stained with DAPI (**a**) and after hybridization with nucleotide polyprobes targeting SCM1 cells (**b**). Arrows indicate cells showing the characteristic peanut-like shape of marine Crenarchaeota[12,15]. Scale bars represent 1 μm. **c**, Transmission electron micrograph of negative-stained cells. Scale bar represents 0.1 μm. **d**, Scanning electron micrograph of Au/Pd-sputtered cells. Scale bar represents 0.1 μm.

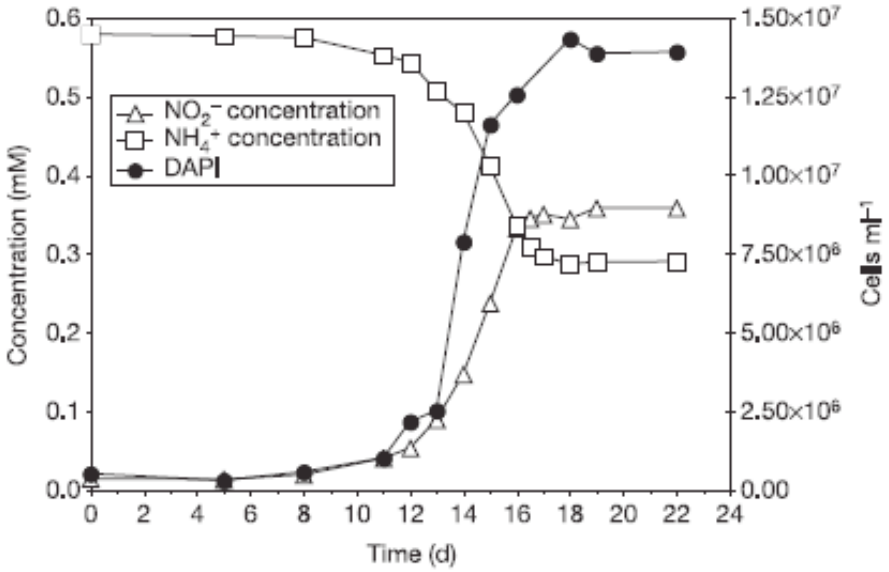


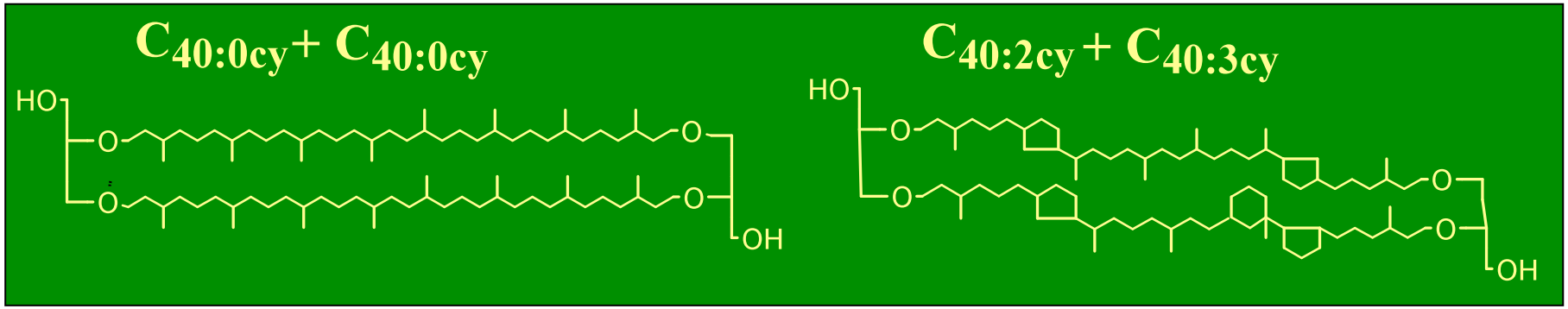

**Figure 3 | Near-stoichiometric conversion of ammonia to nitrite by SCM1.** Growth of SCM1 in Synthetic Crenarchaeota Media containing ammonium chloride and bicarbonate as sole energy and carbon sources, respectively. DAPI-stained cells were directly counted on filters by fluorescence microscopy. Ammonium consumption and nitrite production were determined in triplicate as described previously[27].

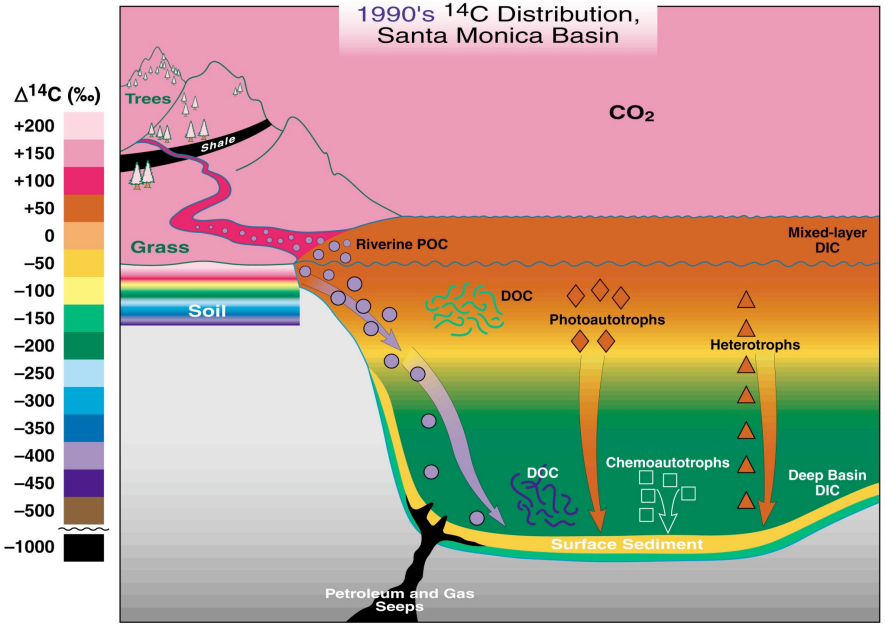
**Nitrosopumilis maritimus**

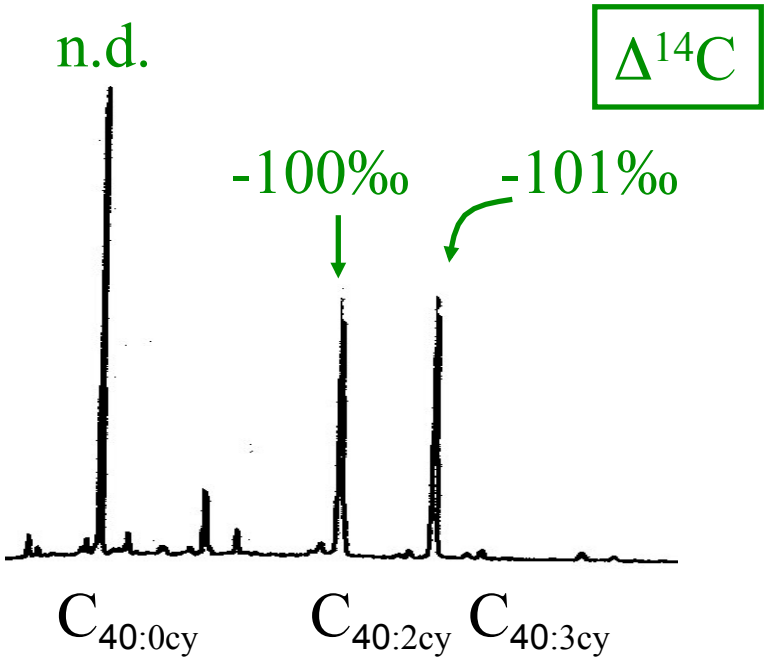
*Könneke et al., 2005*

Slide courtesy of Ann Pearson, Harvard University

Compound specific radiocarbon analysis of lipid tetraethers from
Santa Barbara Basin sediments



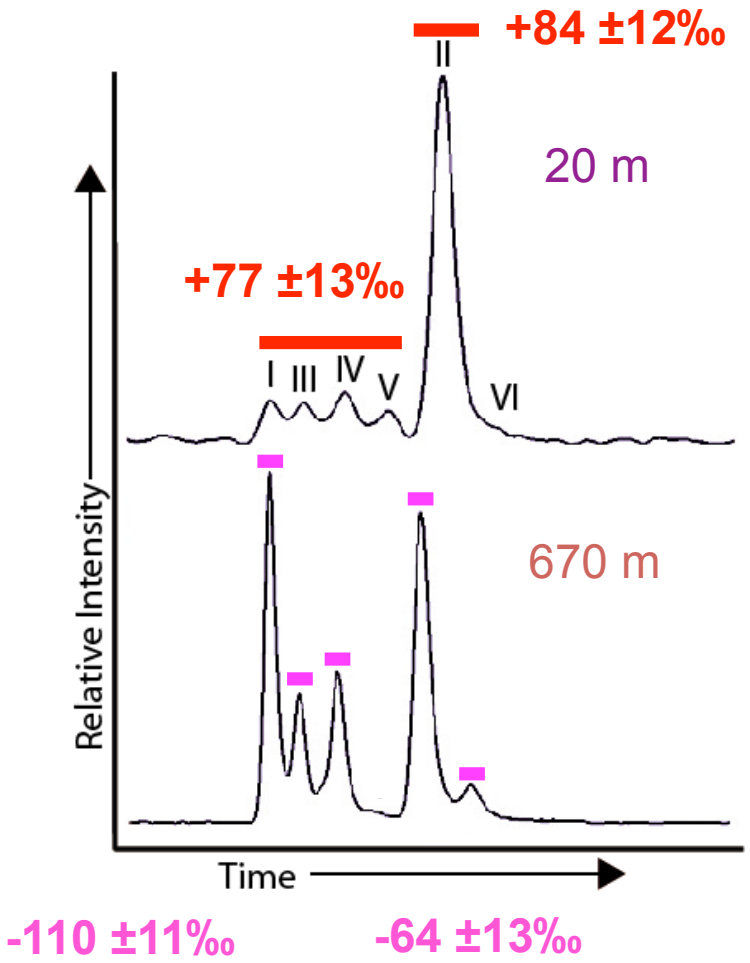

Slide courtesy of Ann Pearson, Harvard University

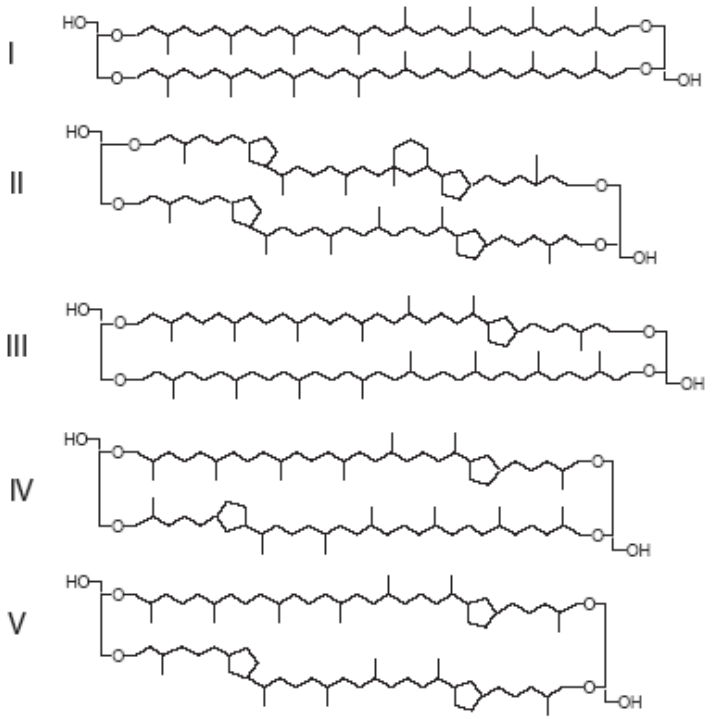
# Quantifying archaeal community autotrophy in the mesopelagic ocean using natural radiocarbon

Anitra E. Ingalls*†‡, Sunita R. Shah*†, Roberta L. Hansman§, Lihini I. Aluwihare§, Guaciara M. Santos¶, Ellen R. M. Druffel¶, and Ann Pearson*‖

*Department of Earth and Planetary Sciences, Harvard University, Cambridge, MA 02138; §Scripps Institution of Oceanography, University of California at San Diego, La Jolla, CA 92093; and ¶Department of Earth System Science, University of California, Irvine, CA 92697

Edited by John M. Hayes, Woods Hole Oceanographic Institution, Woods Hole, MA, and approved March 3, 2006 (received for review November 23, 2005)



## Chemoautotrophic Archaea in the mesopelagic ocean

Head-to-head tetraterpenoids have been measured in lipids for several decades. Known sources included extremophile (hypersaline, hot spring, Hydrothermal vent environments) and methanogenic Archea. Archea were not thought to be abundant in low temperature environments.

Archaeal lipid distributions in contemporary sediments were explained as indicators of widespread methanogenesis in micro-environments (surface seawater is often supersaturated with methane).

It was noted early that these head-to-head tetraterpenoids were isotopically heavy relative to lipids from photoautotrophs – but this was not well explained.

The application of 16sRNA clarified the picture immensely, showing that Archaea were widely distributed in low temperature environments. Archaeal lipid analysis showed this to be the case as well. Isotopic values were explained as arising from heterotrophy on enriched substrates, or chemoautotropic $CO_2$ fixation.

Compound specific radioisotope measurements on archaeal lipids support chemoautrophy, probably associated with nitrification.