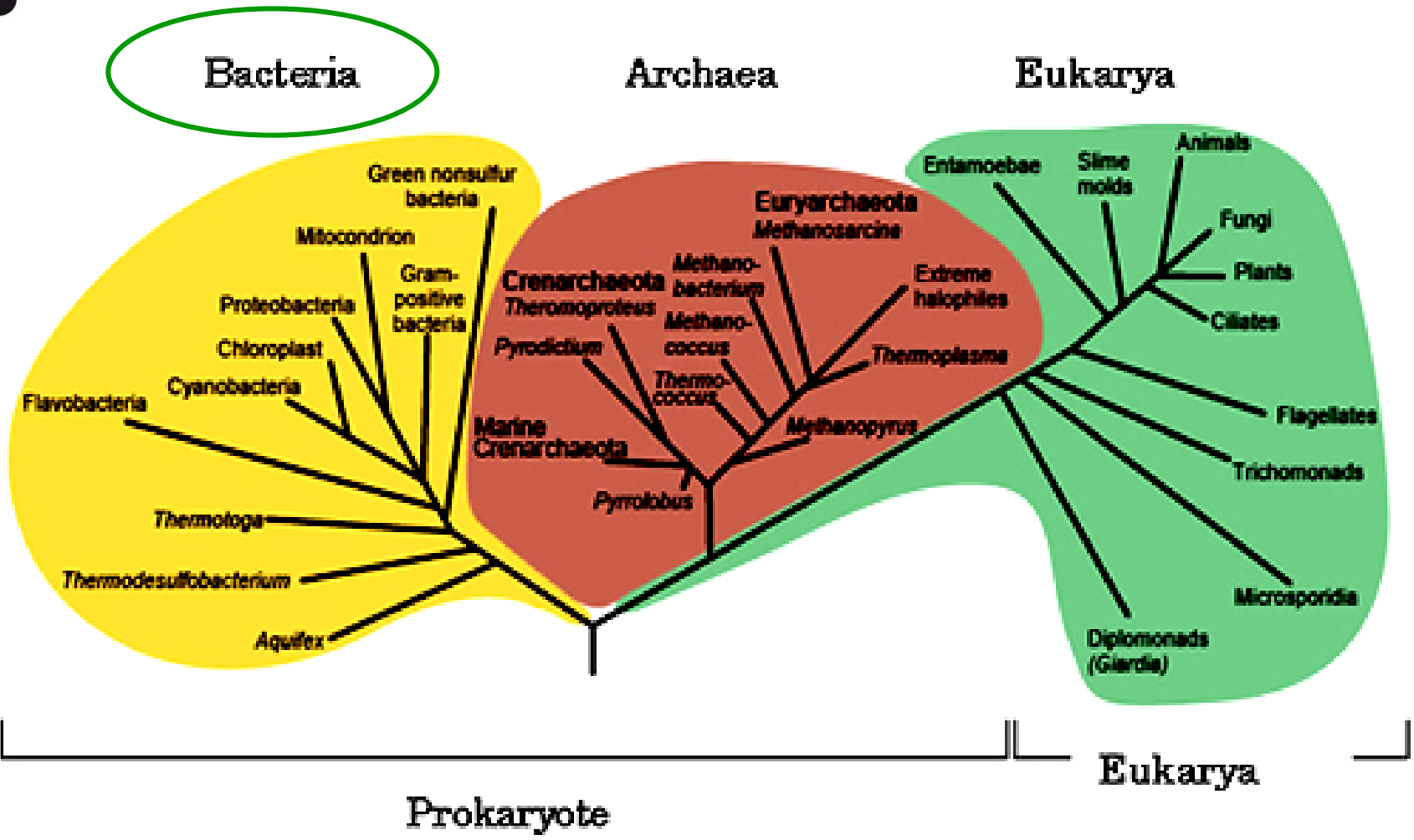
Archaea in the environment

Tree of Life


Bacteria
Archaea
Eukarya
Green nonsulfur bacteria
Mitocondrion
Gram-positive bacteria
Proteobacteria
Chloroplast
Cyanobacteria
Flavobacteria
Thermotoga
Thermodesulfobacterium
Aquifex
Euryarchaeota
Methanosarcina
Crenarchaeota
Thermoproteus
Methano-bacterium
Extreme halophiles
Pyrodictium
Methano-coccus
Thermoplasma
Thermo-coccus
Methanopyrus
Marine Crenarchaeota
Pyrolobus
Entamoebae
Slime molds
Animals
Fungi
Plants
Ciliates
Flagellates
Trichomonads
Microsporidia
Diplomonads (Giardia)
Eukarya
Prokaryote

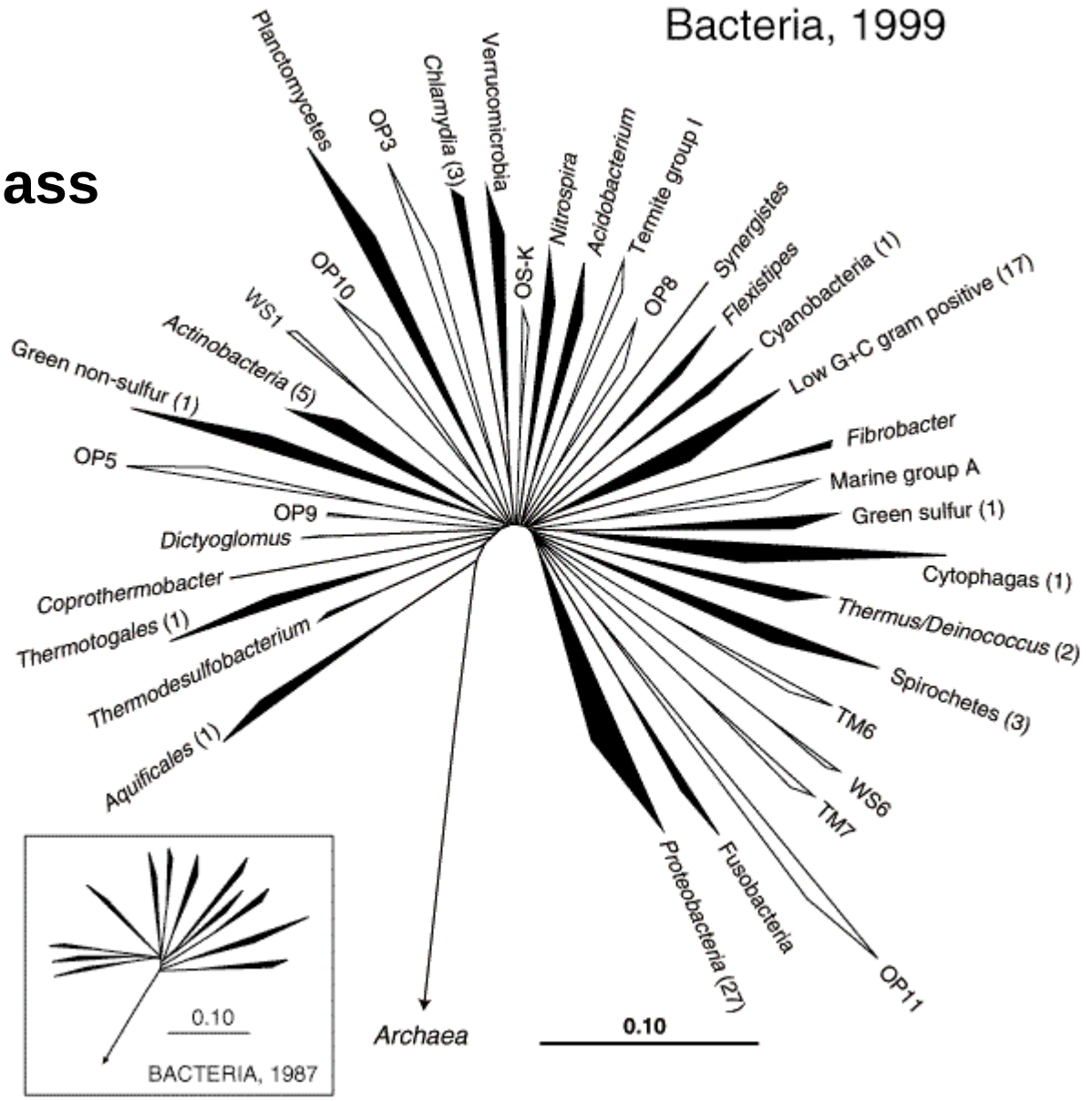
BACTERIA

1987: 12 phyla/class

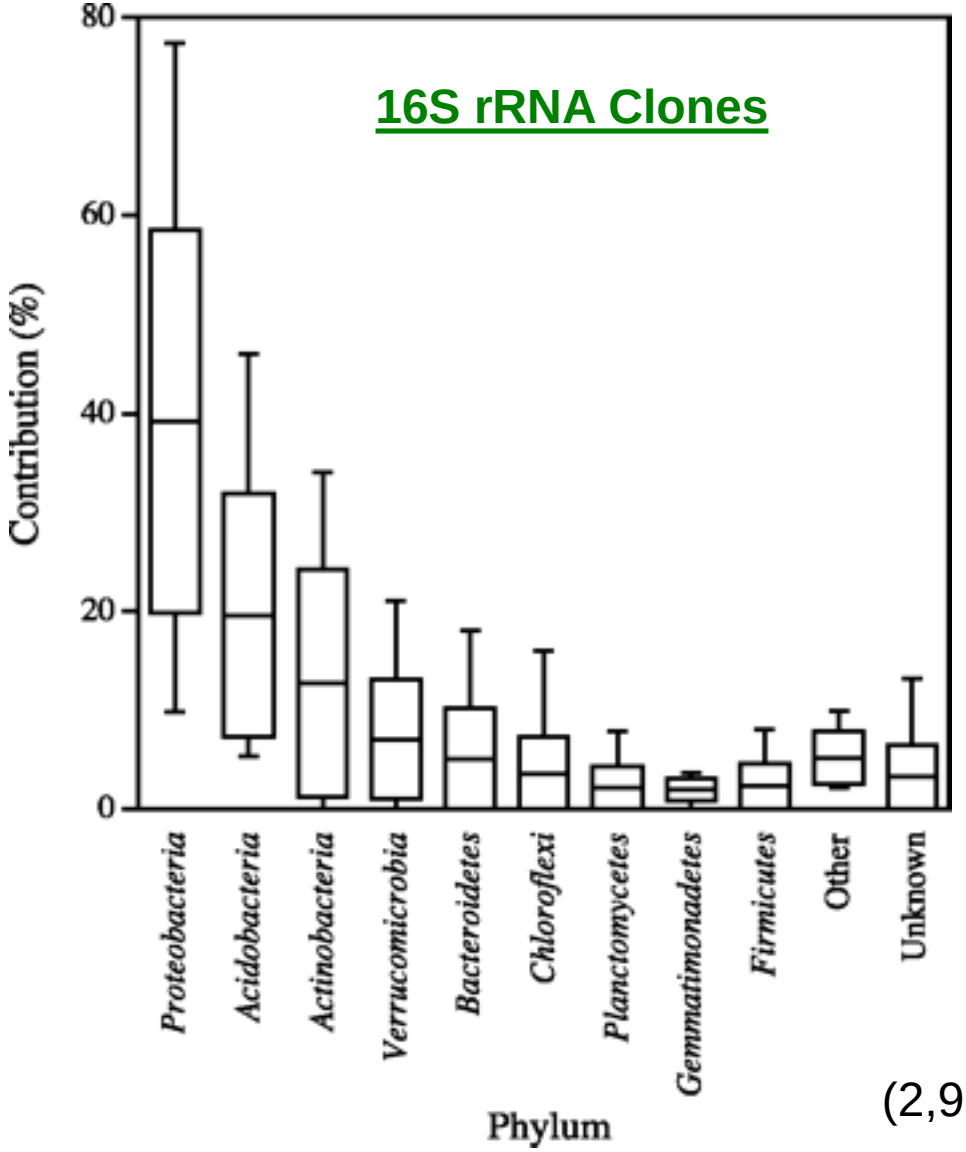
1999: 36

2008: 40

2011: ~50



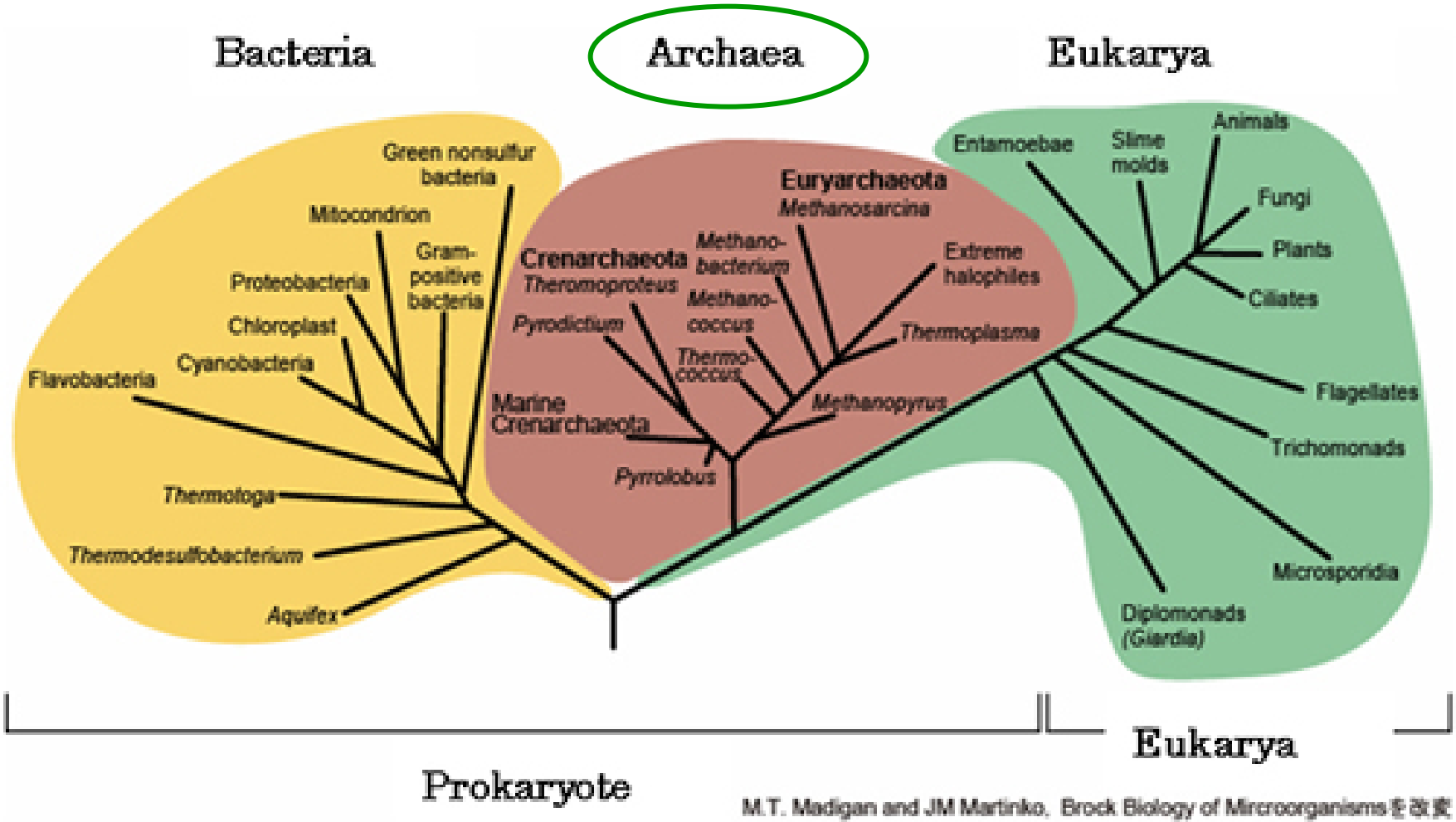
Dominant bacterial phyla in soil



Ranked genomes:

Proteobacteria
Firmicutes
Actinobacteria
Cyanobacteria
Spirochaetes
Bacteroidetes

(2,920 clones, 21 libraries; Janssen 2006).


Bacteria
Archaea
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Green nonsulfur bacteria
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Cyanobacteria
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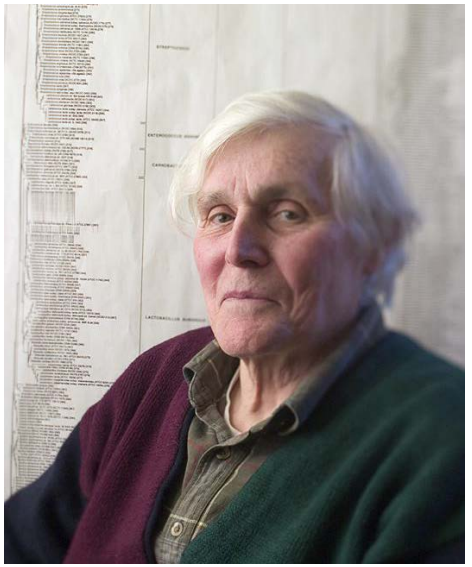
Thermodesulfobacterium
Aquifex
Euryarchaeota
Methanosarcina
Crenarchaeota
Theromoproteus
Methano-bacterium
Methano-coccus
Pyrodictium
Thermo-coccus
Methanopyrus
Marine Crenarchaeota
Pyrolobus
Extreme halophiles
Thermoplasma
Entamoebae
Slime molds
Animals
Fungi
Plants
Ciliates
Flagellates
Trichomonads
Microsporidia
Diplomonads (Giardia)
Prokaryote
Eukarya
M.T. Madigan and JM Martinko, Brock Biology of Mircroorganisms を改変

History

Alessandro Volta found that “combustible air” (methane) is produced in lakes and bogs in the early 1800’s.



Archaea recognized as “the third domain” 1977, discovery by Carl Woese

A universal tree of life

- Relatedness of all organisms
- The origin of life

Evolutionary chronometer?

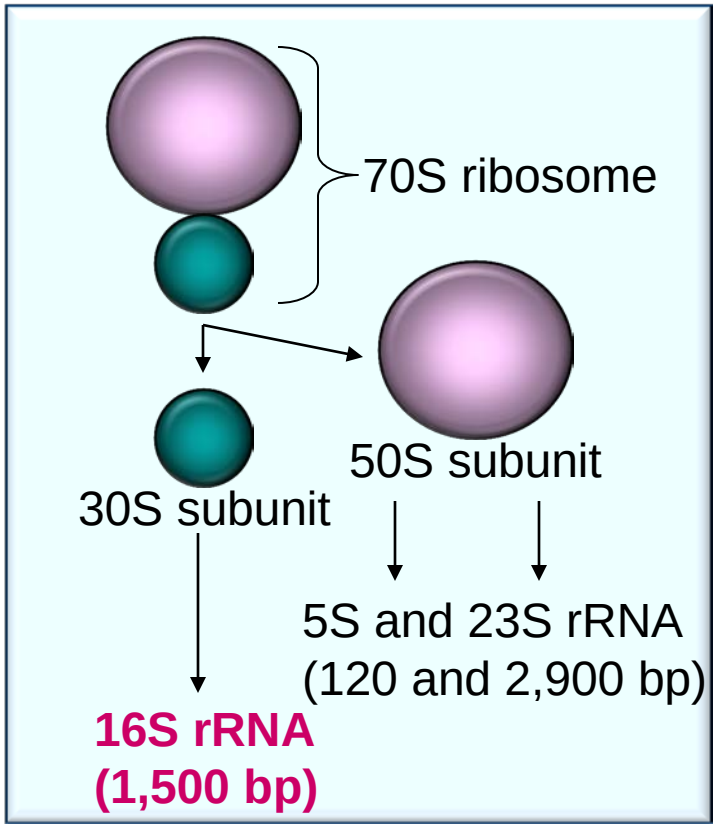
Macro molecules that are used to determine evolution

- **Universal molecule**
- **Sequence comparisons possible (nucleotide or amino acid)**
- **The same function in all organisms**

The rate of evolution in the molecule must correspond to the evolutionary distance between the organisms.

Ribosomal RNA genes

- **Likely antiquity of protein-synthezising machinery**
- **Pioneering work by Carl Woese in 70's**



16S rRNA in prokaryotes

(*18S rRNA in eukaryotes*)

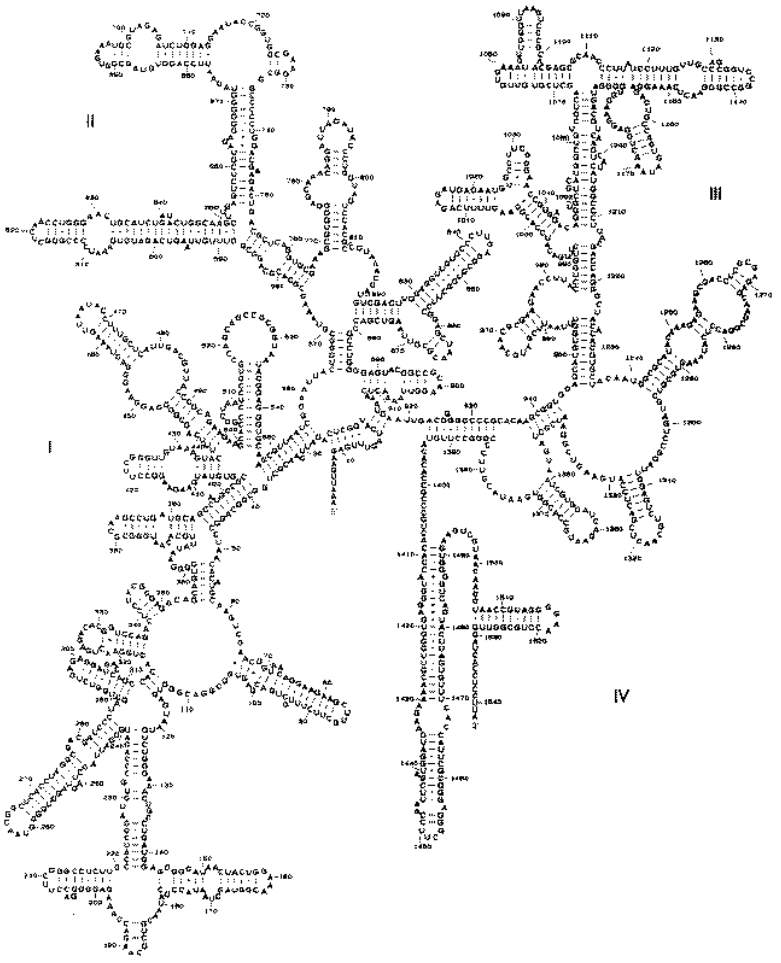
16S rRNA

Universally conserved regions

Semi-conserved regions

Variable regions

Signature sequences **can be used to design molecules that target different regions to ”catch” certain species, genera, families etc.**



Prokaryotes

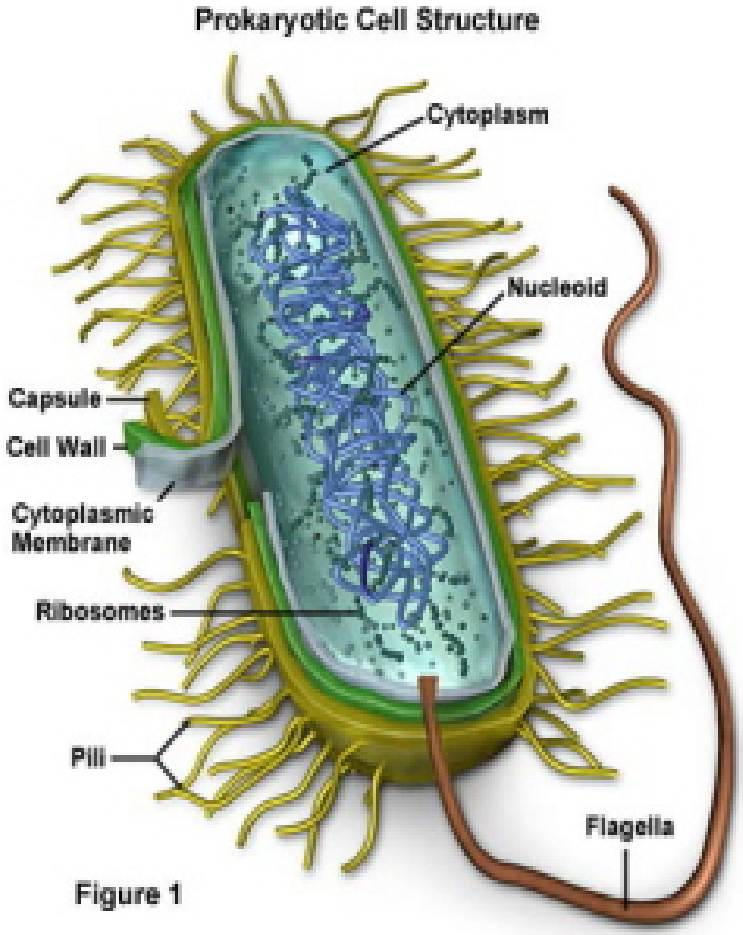


Figure 1

Prokaryotes are single celled organisms that do not have a nucleus, mitochondria or any other membrane bound organelles.

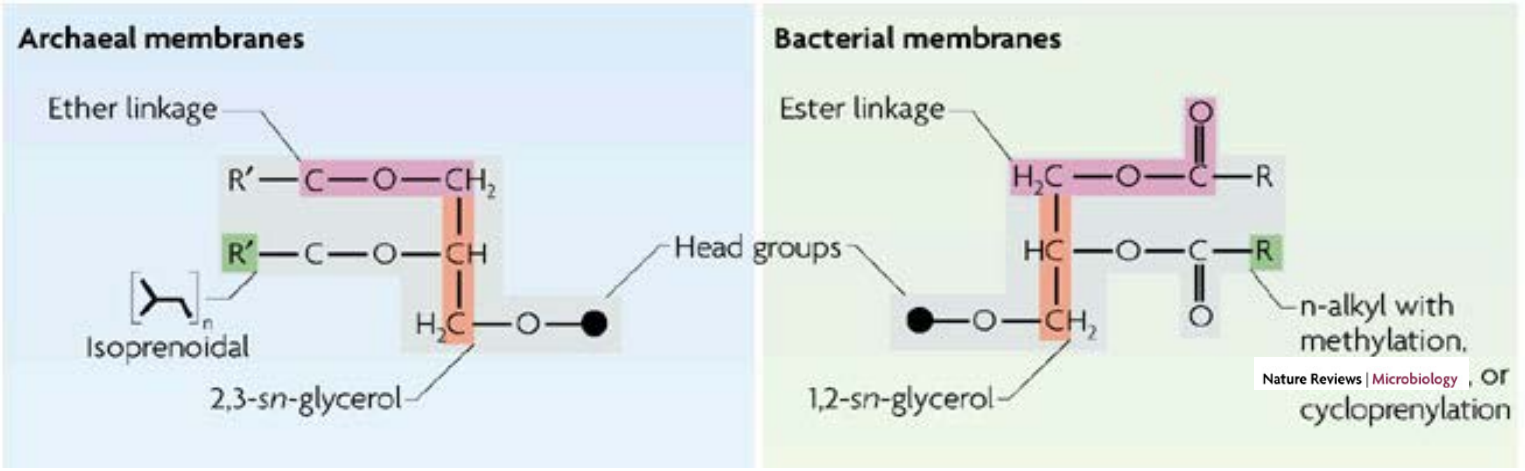
Instead everything is openly accessible within the cell, some free floating, some bound to the walls of the cell membrane,

Prokaryotes come in two sorts, *Archaea* and *Bacteria*.

As different, if not more different, from each other, than they are from protozoans, fungi, plants and us.

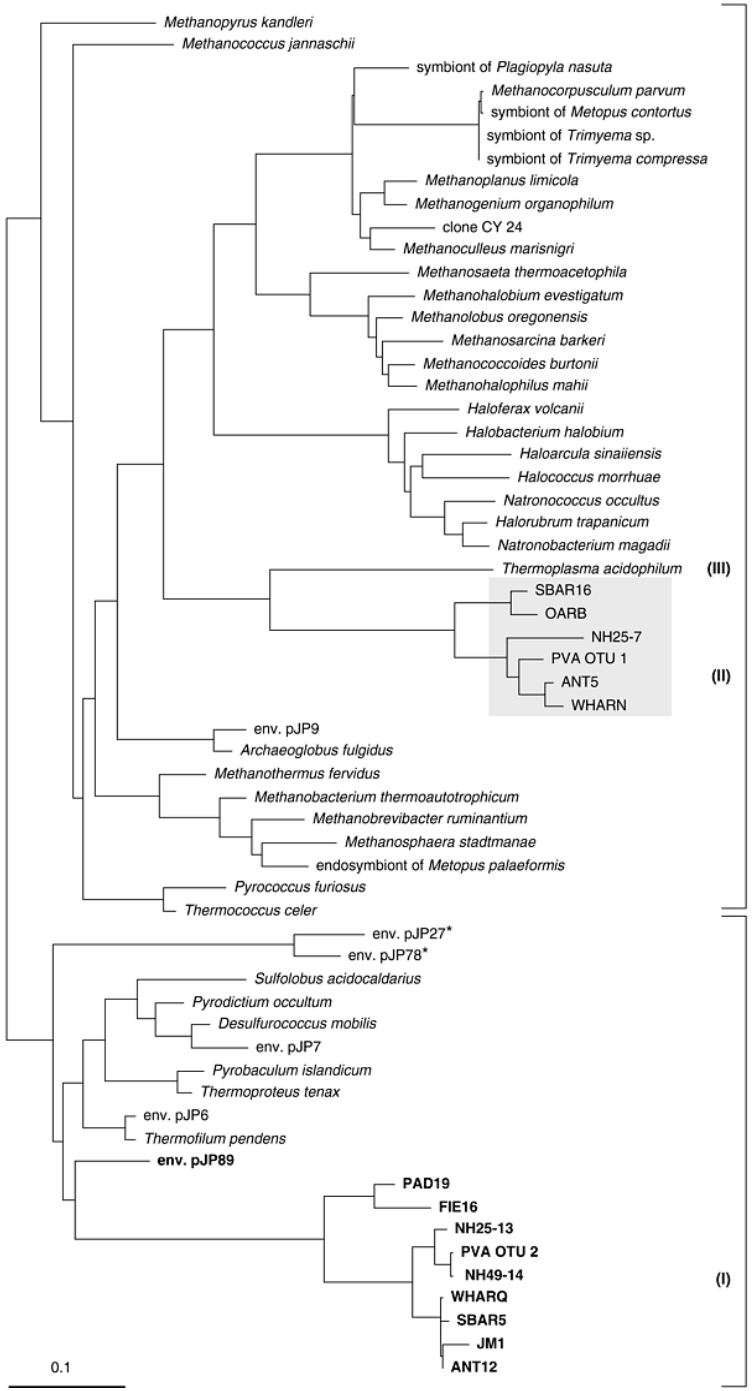
Bacterial vs Archaeal properties

- Glycerol-1-phosphate instead of glycerol-3-phosphate in membranes
- Lipids ether-linked to glycerol



- Despite the fact that the archaea are prokaryotes, the information machinery (replication, transcription, recombination, repair, translation) is homologous to that of eukaryotes

- Unique metabolic pathways (e.g. methanogenesis) among Archaea
- Most extremophilic species are archaea (but majority of archaea are not)



Two main branches (phyla)

Euryarchaeota

Crenarchaeota

Additional phyla debated:

”Korarchaeota”
”Thaumarchaeota”

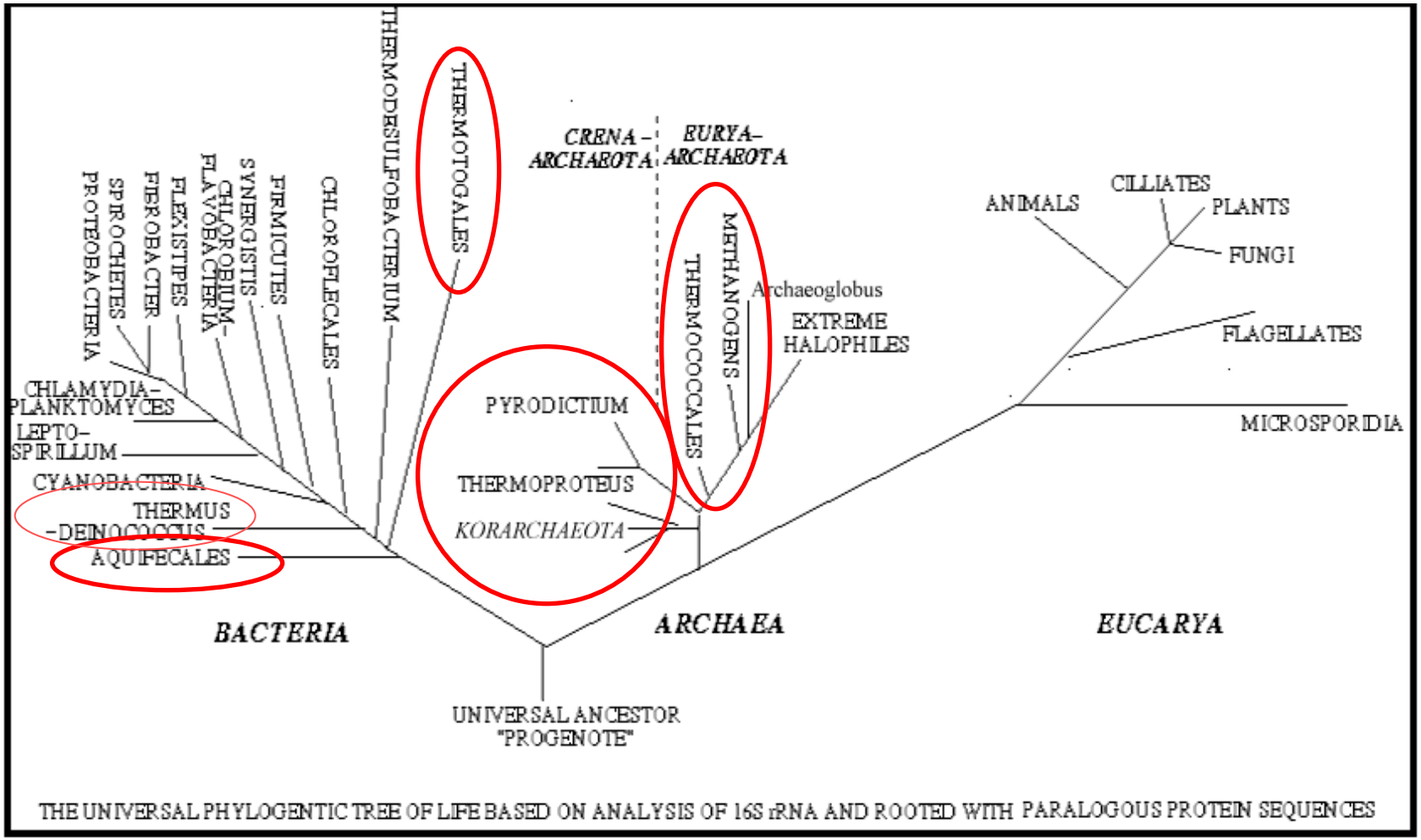
(”Nanoarchaeota”
probably not phylum)

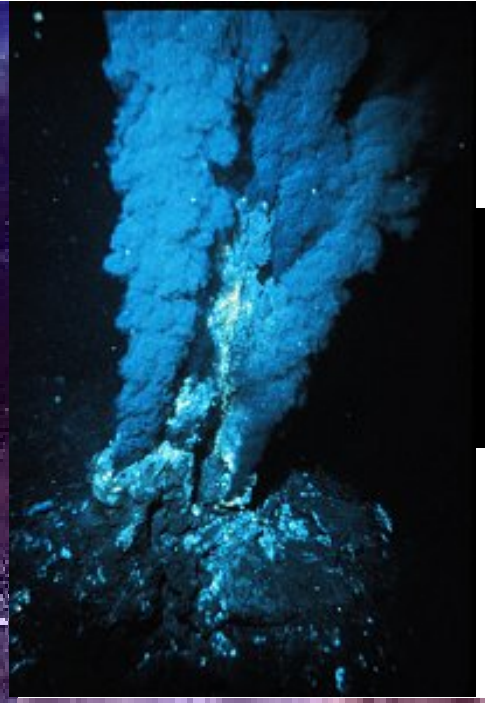
Archaea in soil

Archaea in soil

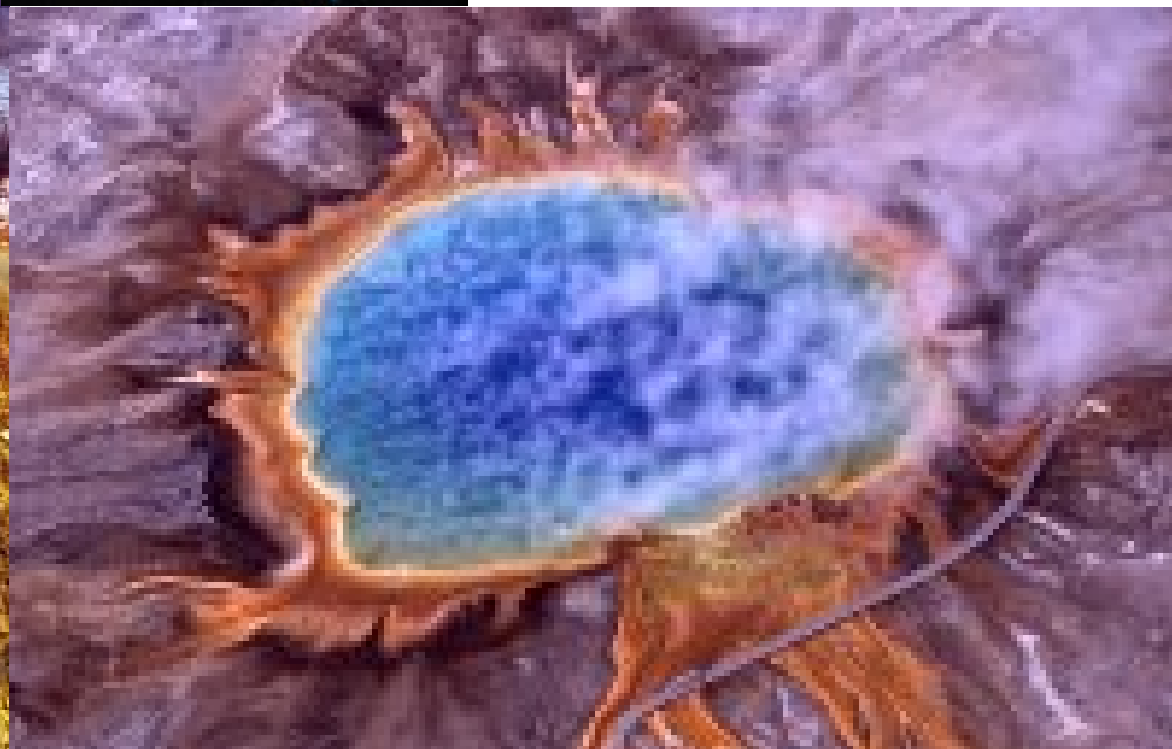
- **Research on the diversity of "non-extreme" Archaea in soil is a rather new sector**
- **Discovery of the novel Crenarchaeota in Finnish forest soil (1996).**
- **Crenarchaeal communities are always found in grassland soils in high numbers, where their ecological function is unknown**
- **Archaea account up to 10% of the microbial biomass in arable soils**

Hyperthermophiles

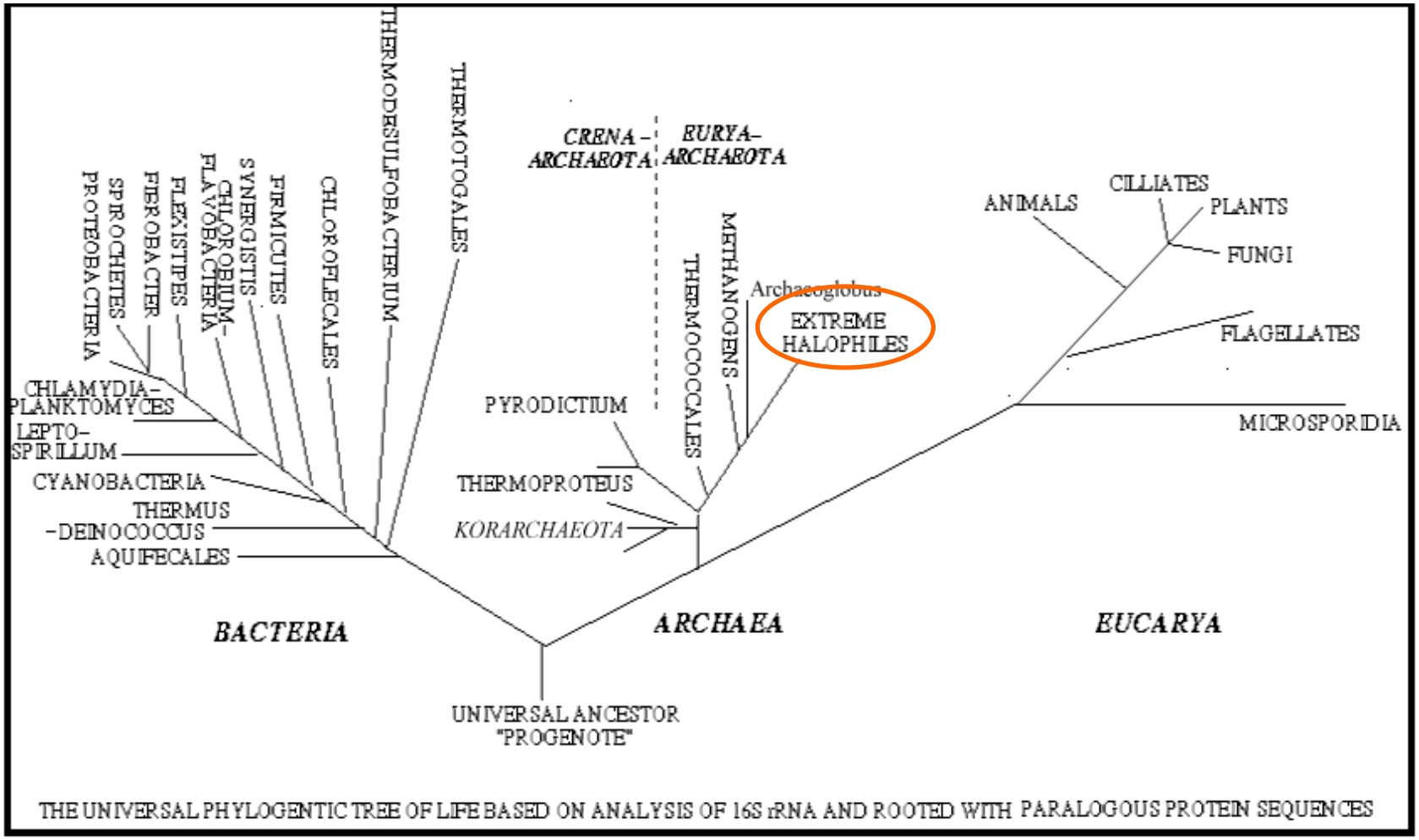




Hot springs



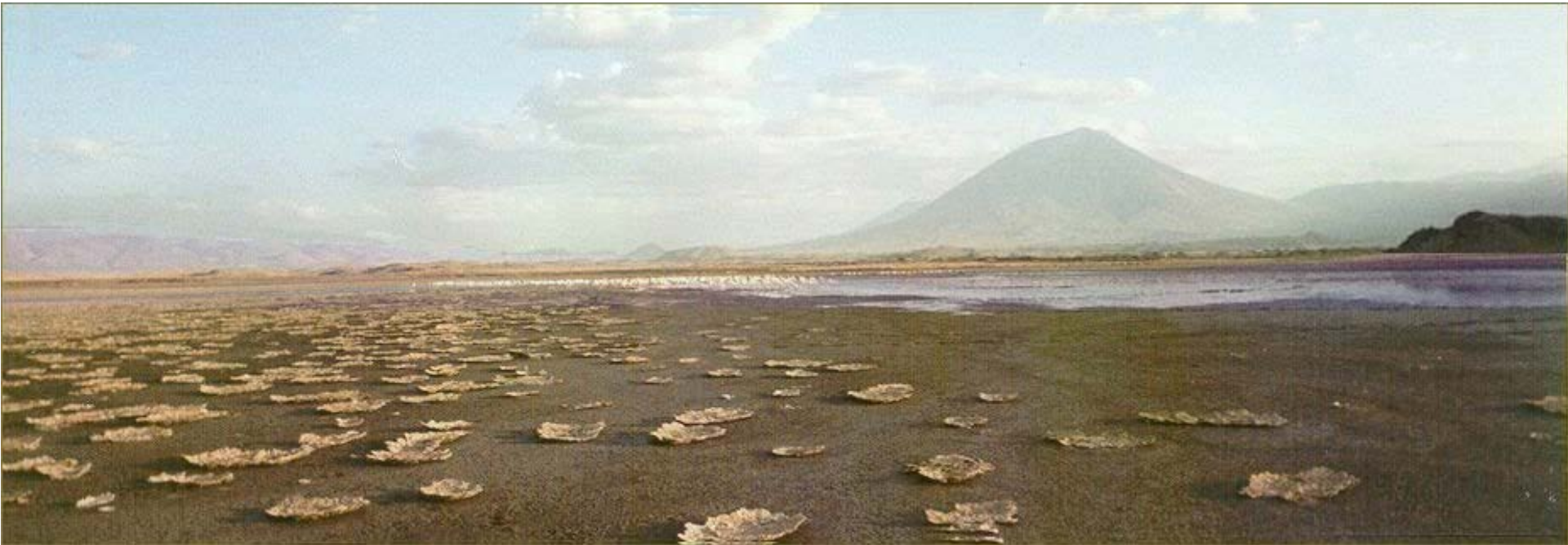
Extreme halophiles



Halophilic blooms

Alkalophiles

- Organisms growing optimally at pH 8.5 – 11
- Very widely distributed in the environment
- Archaea prevalent in haloalkaline (soda) lakes (*Natronococcus, Natronobacterium*)
- Include bacteria (*Bacillus alkalophilus, B. firmus*), fungi, yeasts



Archaea – main physiological groups

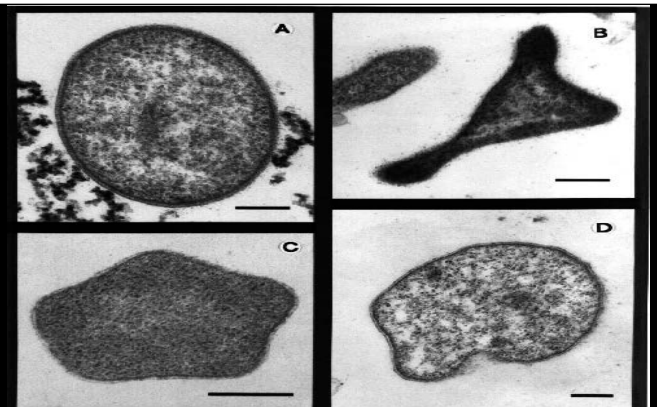
Archaea are often (but majority are *not*) extremophiles:
Anaerobes, thermophiles, acidophiles, alkalophiles, halophiles etc.

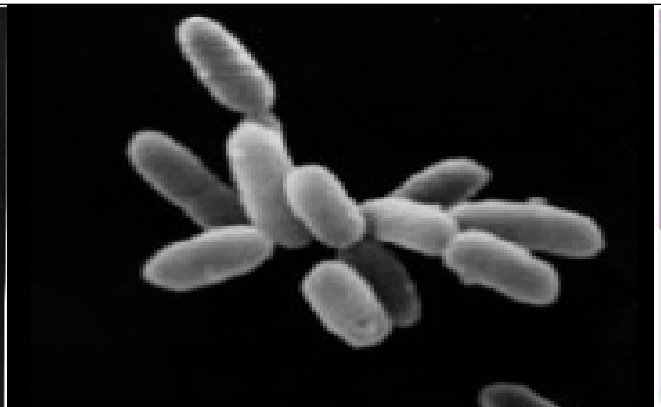
Euryarchaeota

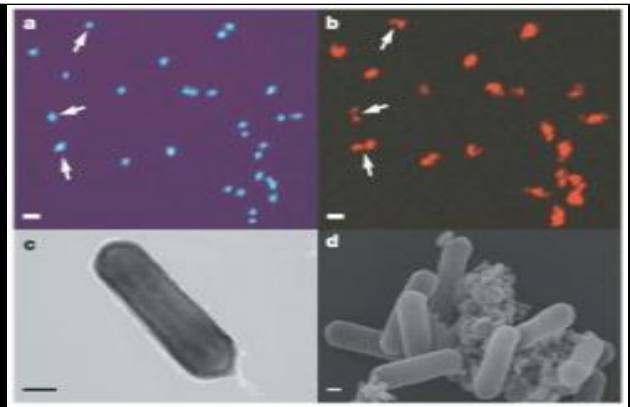
- Halophiles
- Methanogens
- (Hyper-)thermophiles
- Low-temperature aquatic and terrestrial species

Crenarchaeota

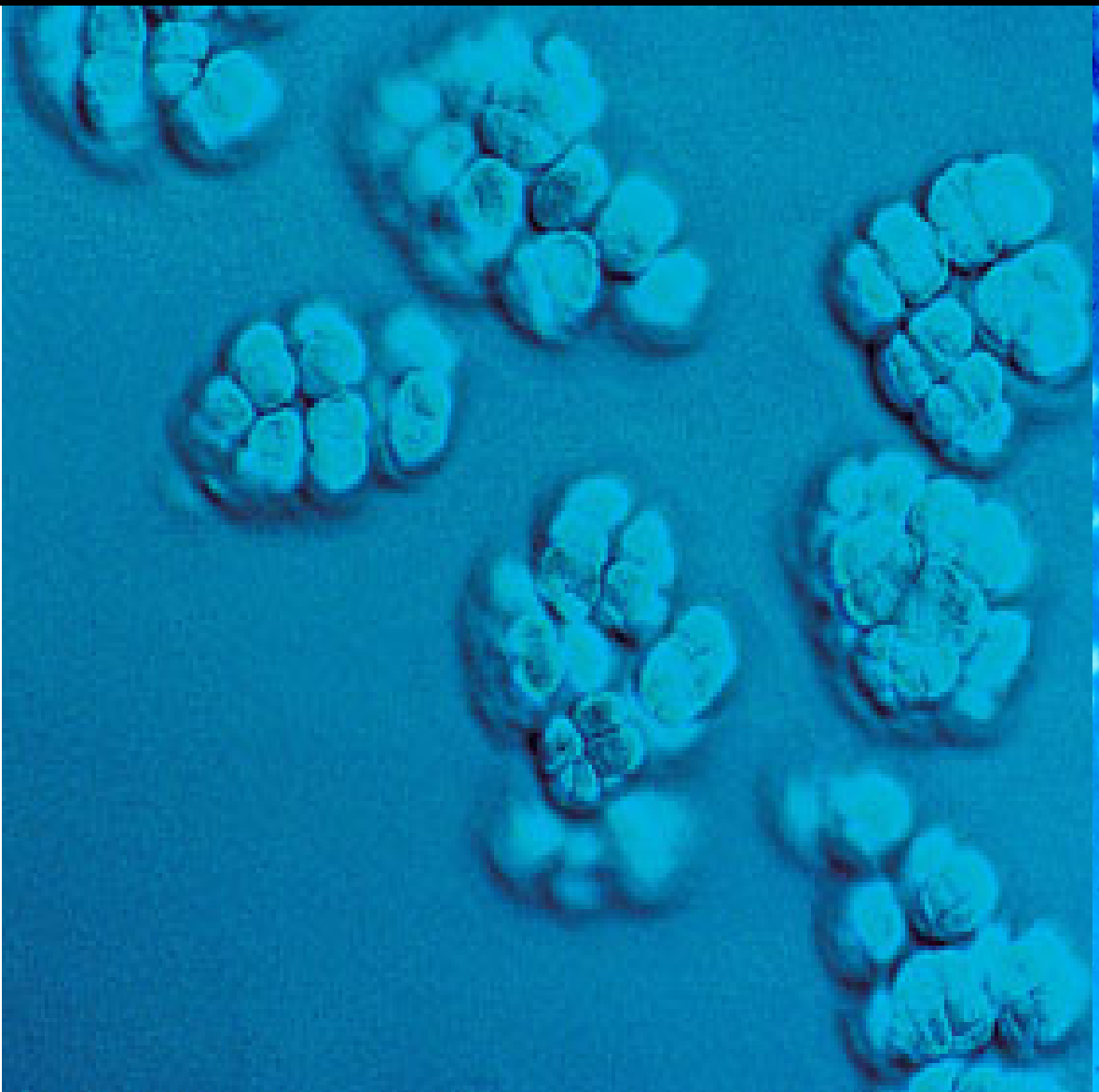
- (Hyper-)thermophiles
- Low-temperature aquatic and terrestrial species

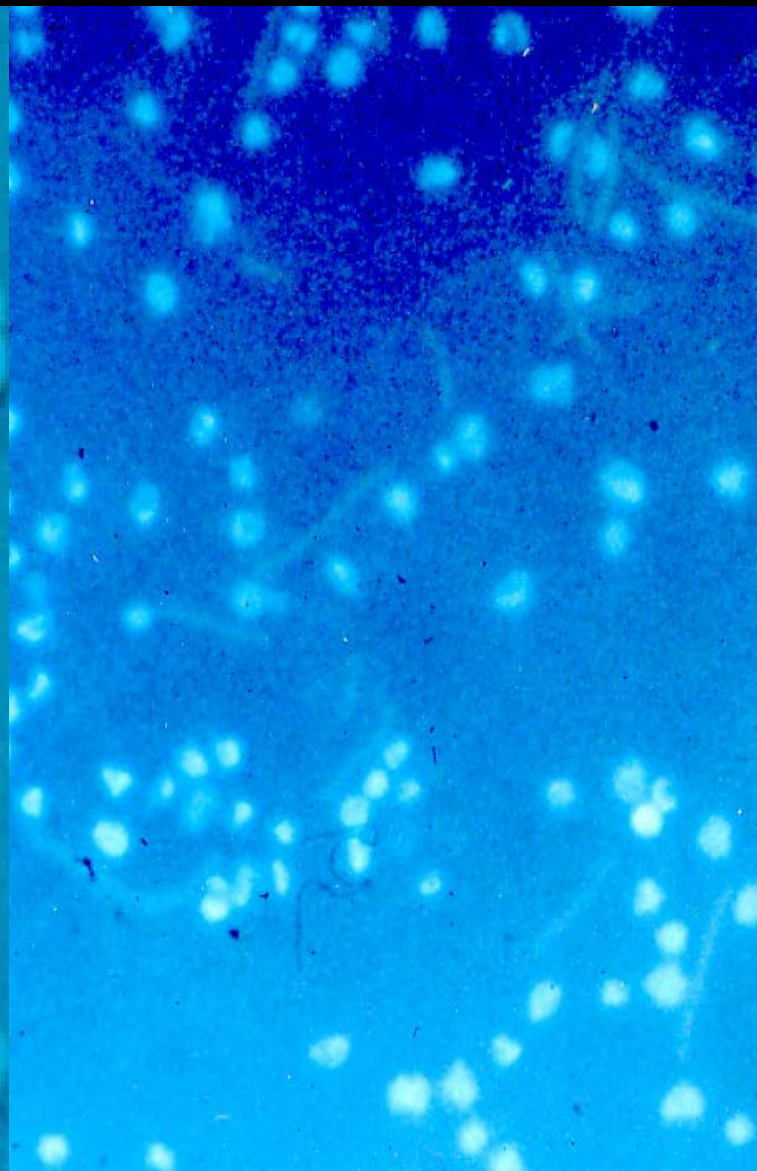




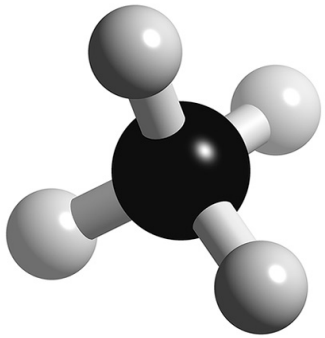


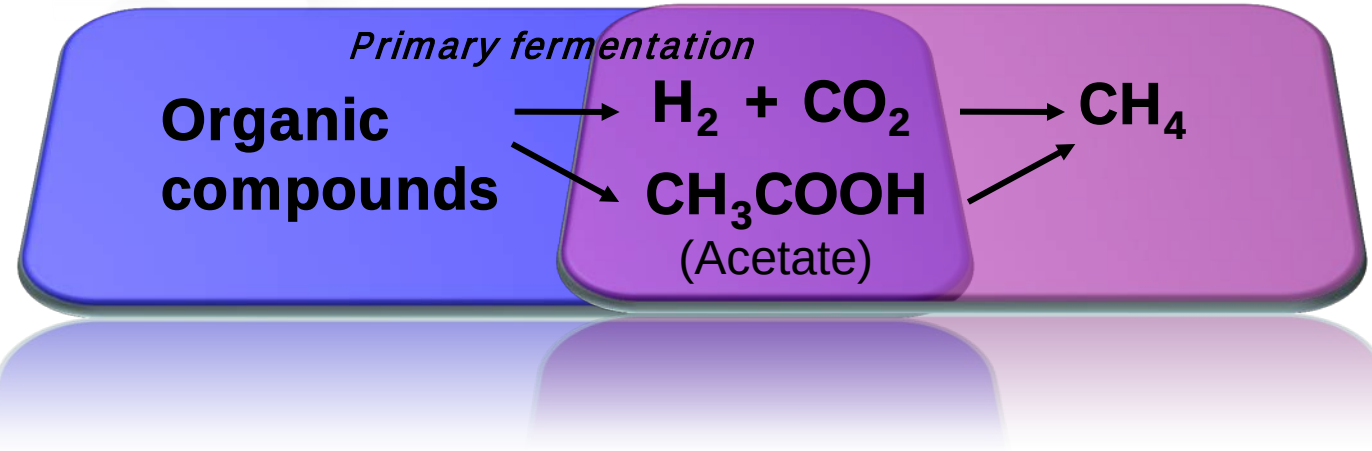
Methanogens

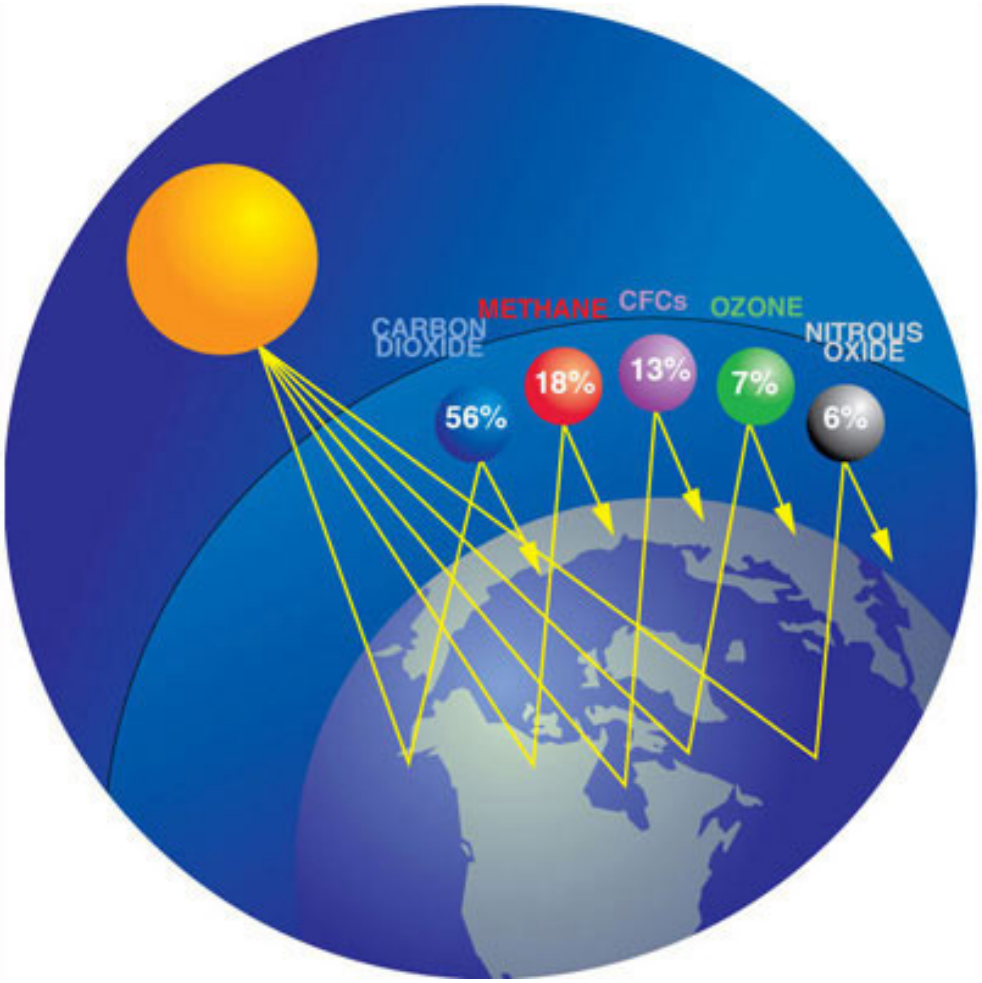




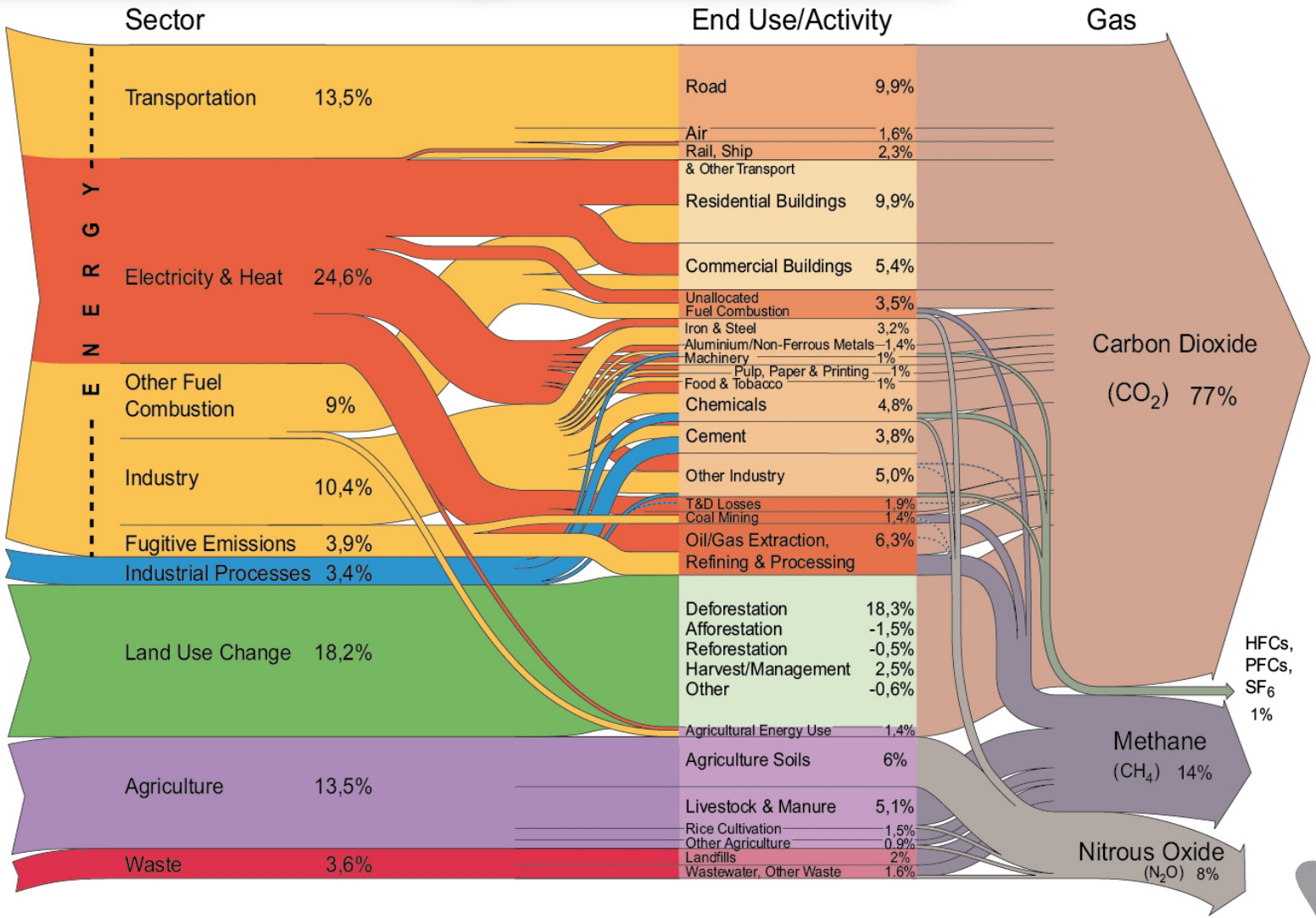
CH_4 producing processes






CARBON DIOXIDE
METHANE
CFCs
OZONE
NITROUS OXIDE
56%
18%
13%
7%
6%

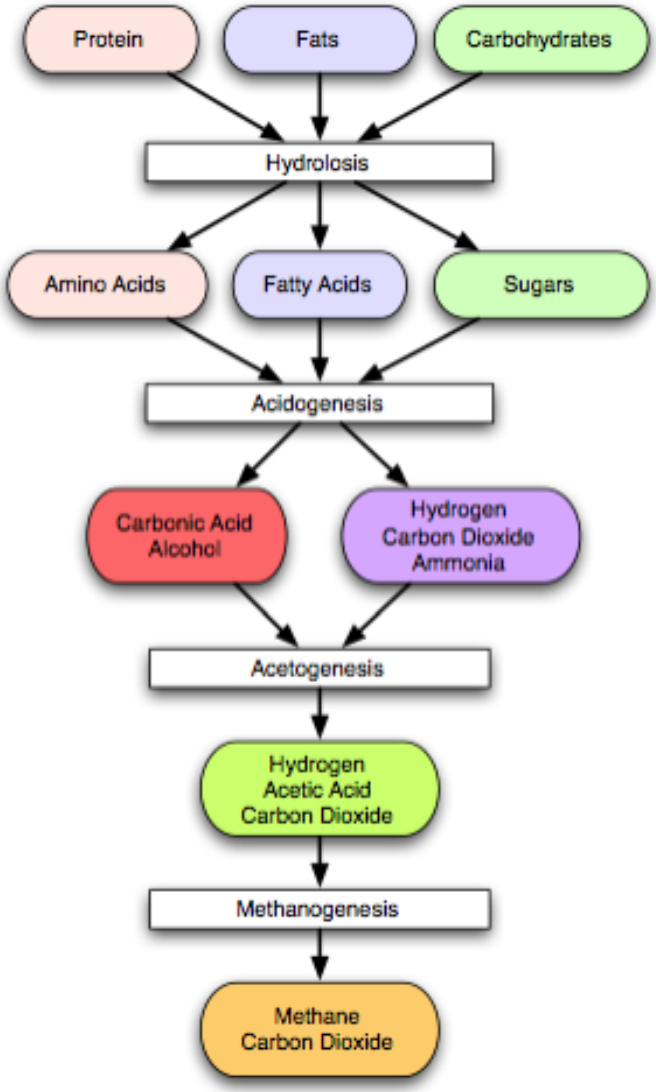
GHG emissions by sector



All data is for 2000. All calculations are based on CO_2 equivalents, using 100-year global warming potentials from the IPCC (1996), based on a total global estimate of 41 755 $MtCO_2$ equivalent. Land use change includes both emissions and absorptions. Dotted lines represent flows of less than 0.1% percent of total GHG emissions.

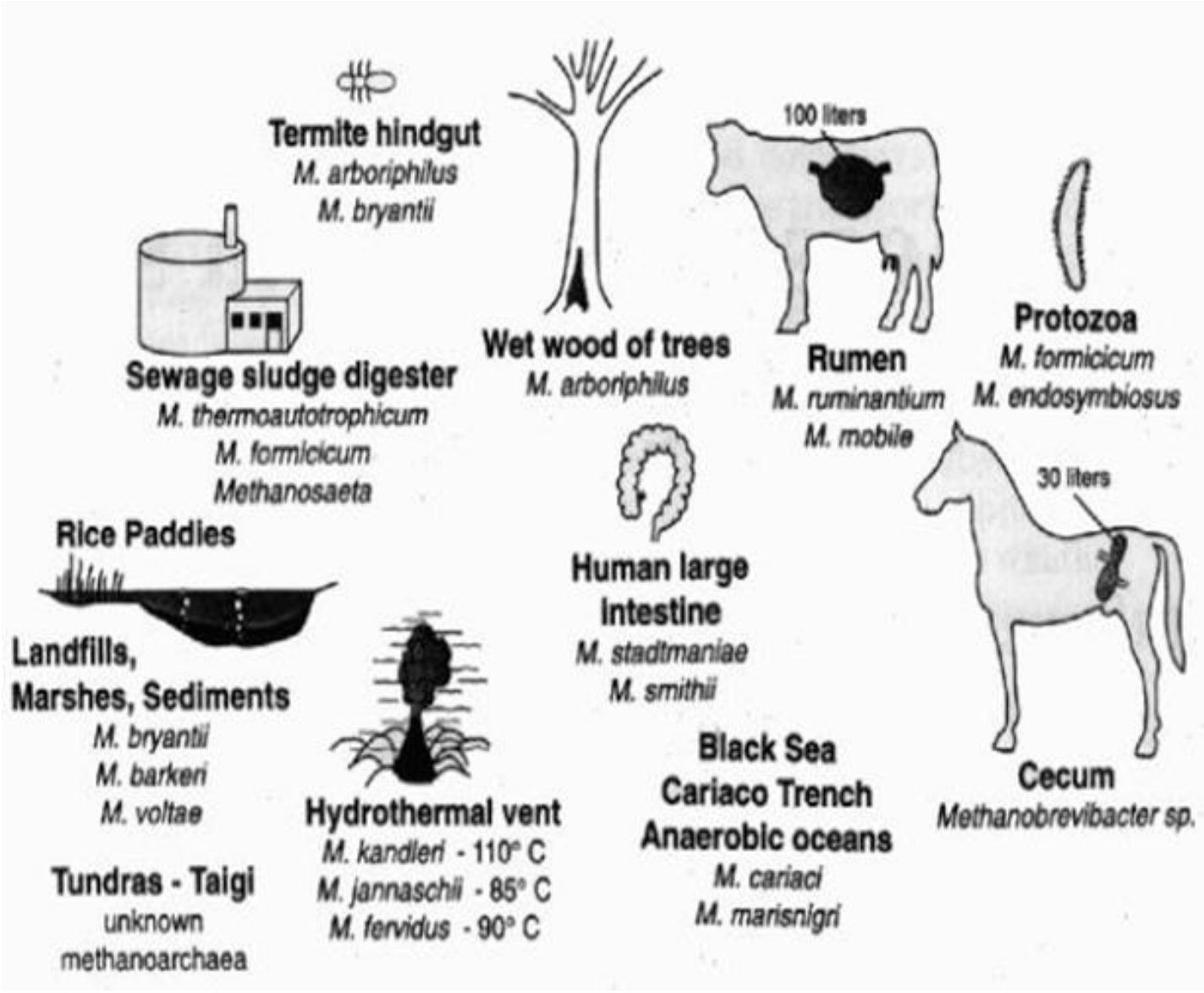
SLU

Anaerobic digestion to methane

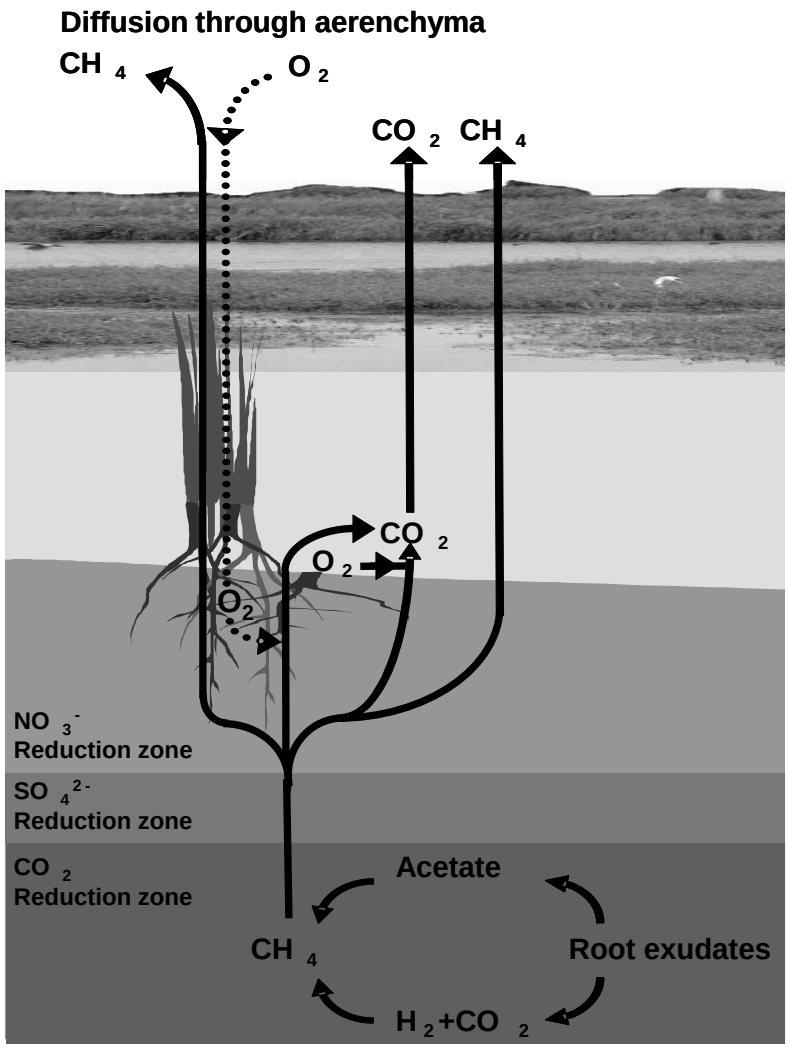


Rice fields

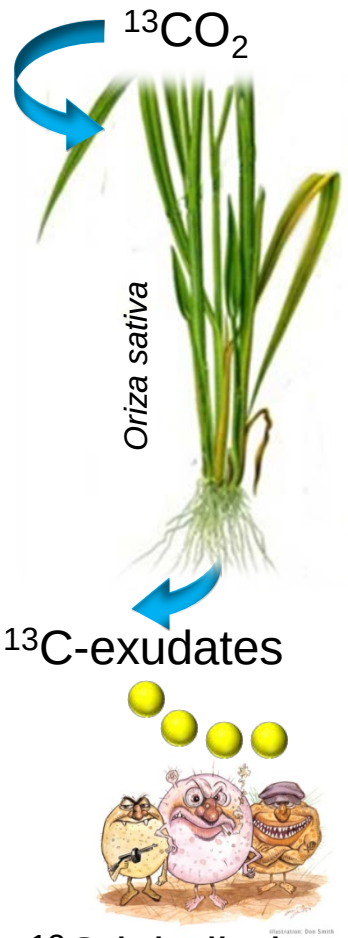
Ruminating animals

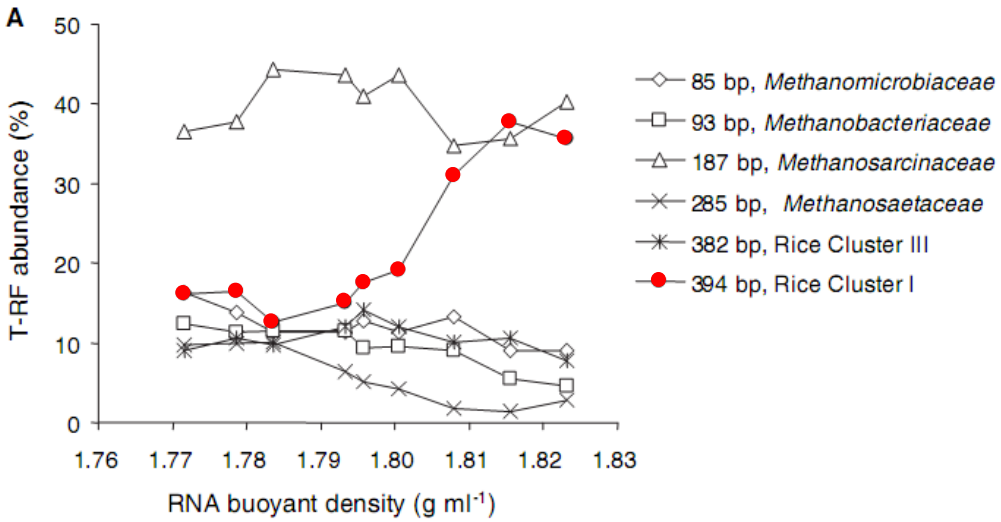

Termite hindgut
M. arboriphilus
M. bryantii
Wet wood of trees
M. arboriphilus
100 liters
Rumen
M. ruminantium
M. mobile
Protozoa
M. formicicum
M. endosymbiosus
Sewage sludge digester
M. thermoautotrophicum
M. formicicum
Methanosaeta
Rice Paddies
Human large Intestine
M. stadtmaniae
M. smithii
30 liters
Cecum
Methanobrevibacter sp.
Landfills, Marshes, Sediments
M. bryantii
M. barkeri
M. voltae
Hydrothermal vent
M. kandleri - 110° C
M. jannaschii - 85° C
M. fervidus - 90° C
Black Sea
Cariaco Trench
Anaerobic oceans
M. cariaci
M. marisnigri
Tundras - Taigi
unknown
methanoarchaea

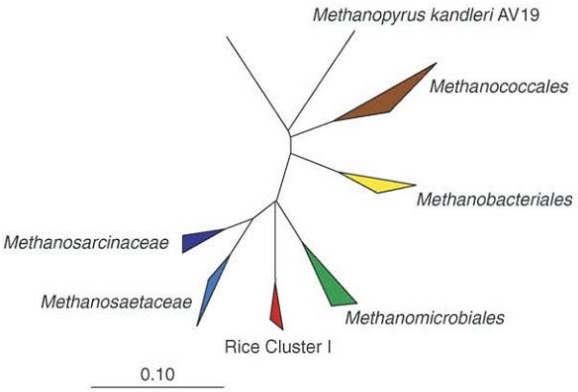
Methanogens in wetlands and rice paddies



Identification of key players







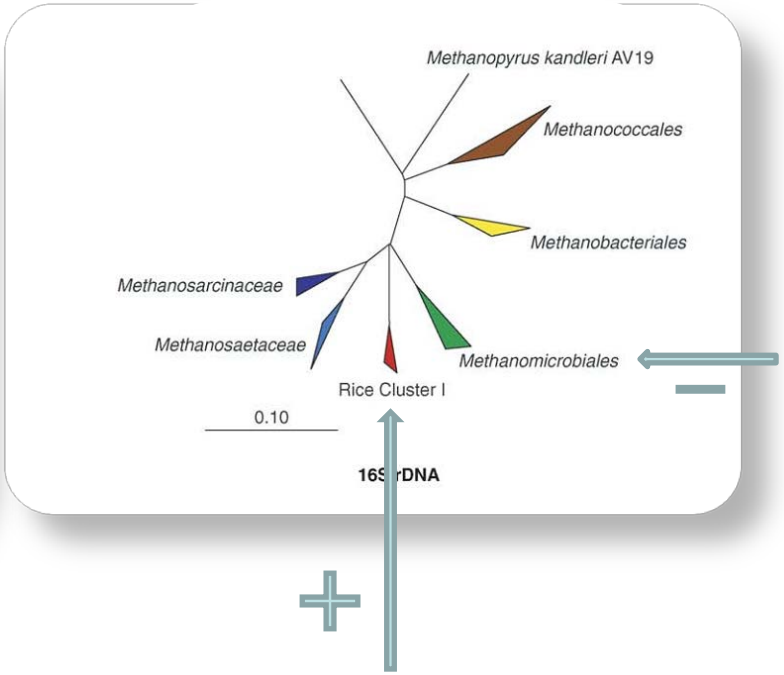
In Situ Stable Isotope Probing of Methanogenic Archaea in the Rice Rhizosphere

Yahai Lu[1,2] and Ralf Conrad[2]*

12 AUGUST 2005 VOL 309 SCIENCE

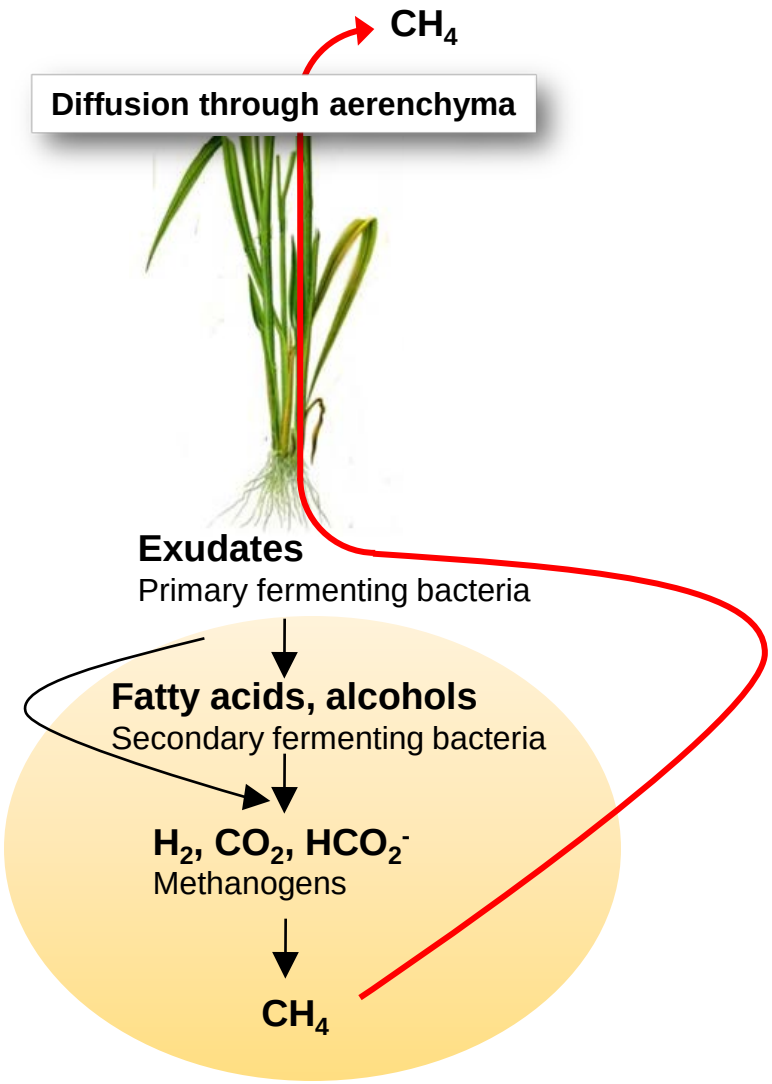
Selection matters – some types emit more methane

- Soil types select type of methanogens colonizing rice roots
- Type of methanogens result in differences in CH_4 emissions



Conrad et al., 2008
Global Change Biology

Methane mitigation strategies



- There are 90,000 known rice cultivars with large variations in genotype and phenotype
- Differences in CH_4 emissions between cultivars that can reach up to 500%

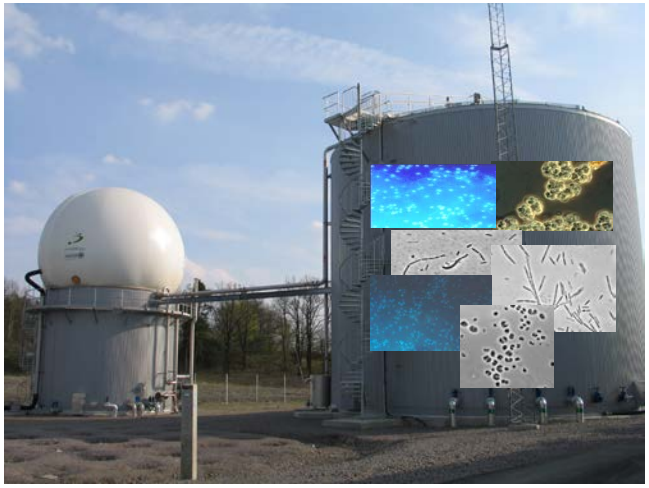
Selection of rice genotypes for lower methane emission

Gogoi *et al*. 2008. Agron. Sustain. Dev. **28:** 181–186

Biogas (CH_4 and CO_2) production:

Waste and crop materials as substrate for biogas producing organisms

Biogas for production of heat, electricity of vehicle fuel



Archaeal N-fixation

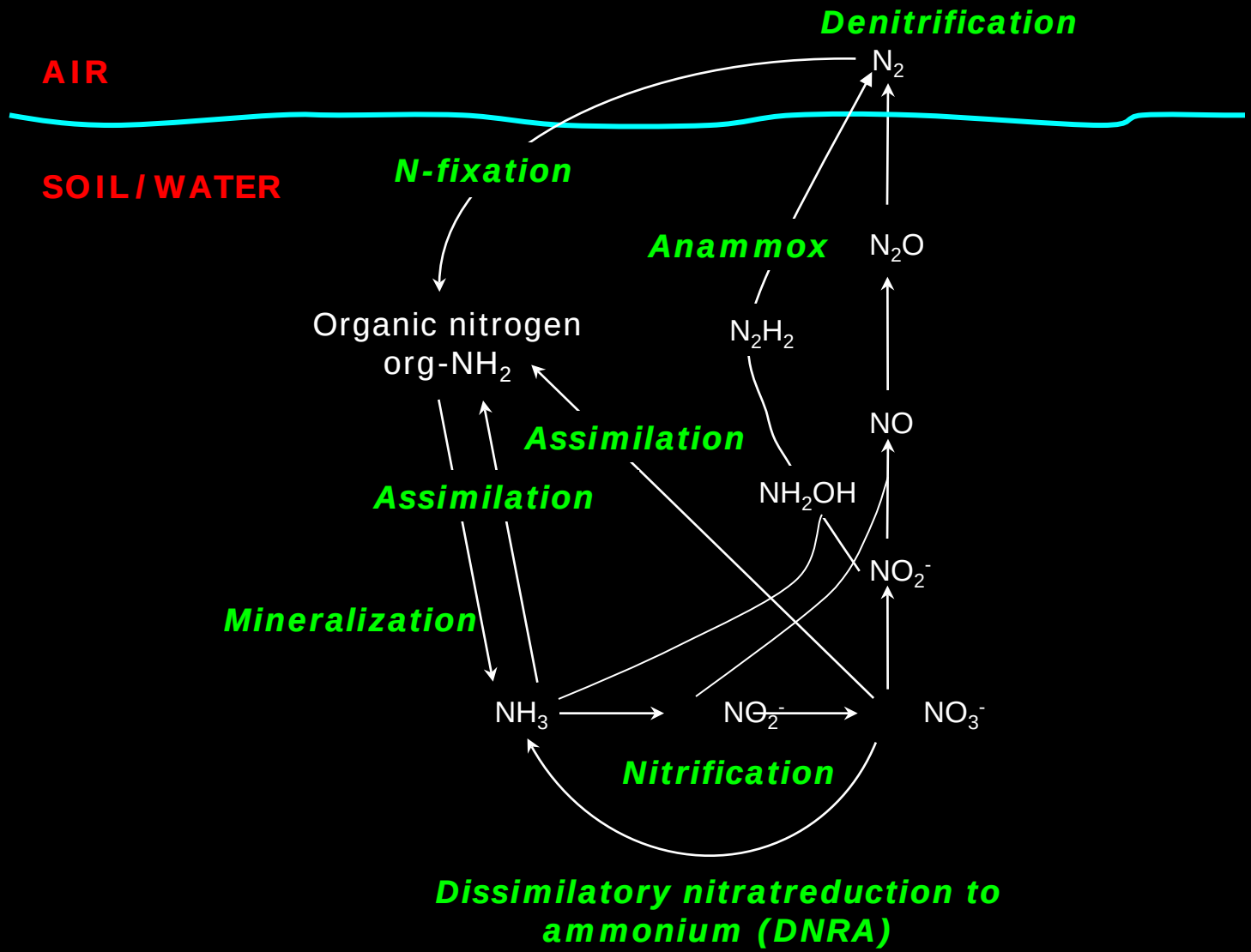
A heat-loving archaeon capable of fixing nitrogen at 92 ºC discovered 2006.

Earth's earliest lineages of organisms capable of N-fixation.

Preceding the kinds of bacteria today's plants and animals rely on to fix N.

Nitrogenase is possibly derived from a nitrogenase present in the last common ancestor of modern life.

Bacteria *and* Archaea in N-cycling



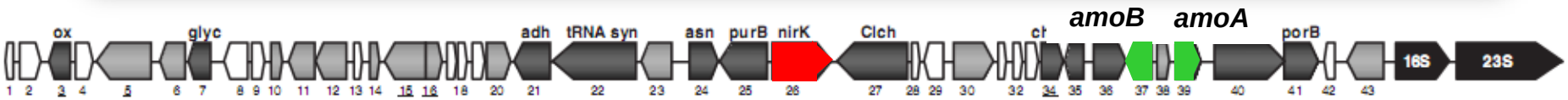
Archaea in nitrification

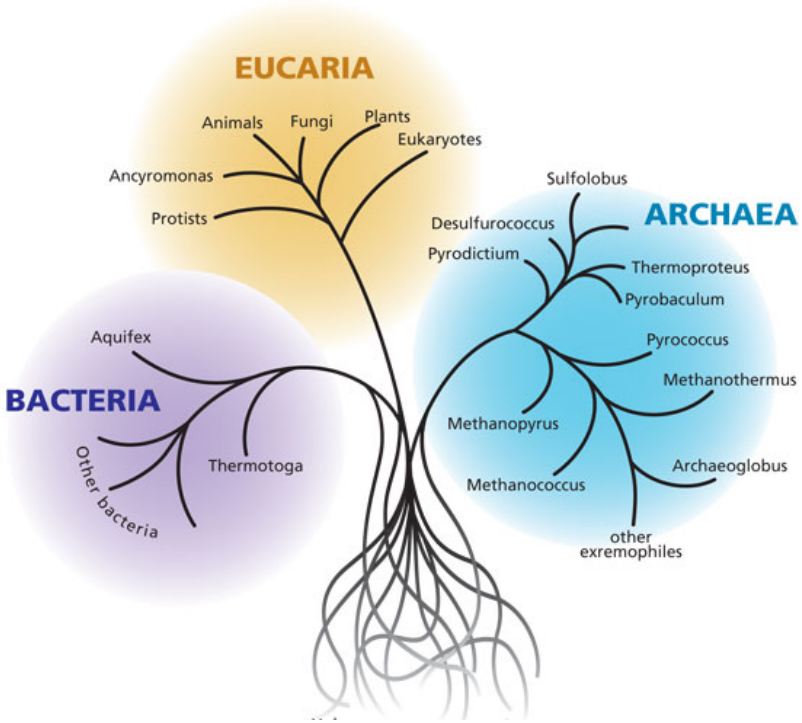
Recent findings include:

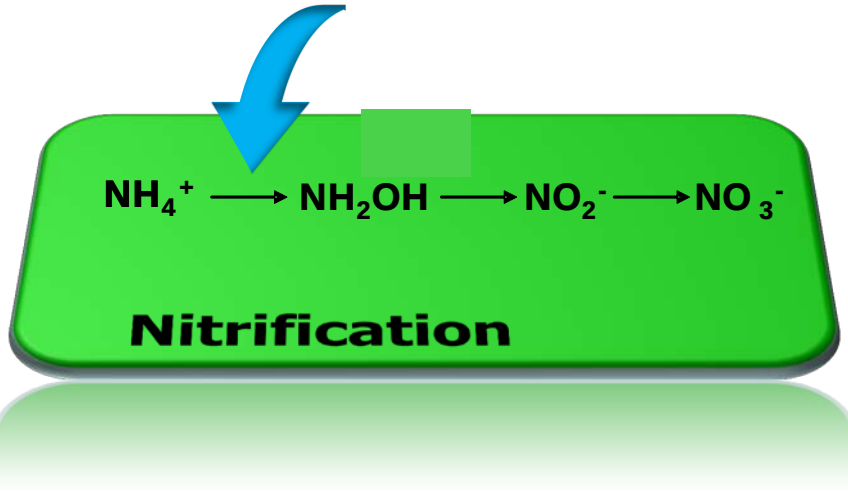
2004: *amoA* gene coding for ammonia oxidation in Archaea in Sargasso sea, suggests ammonia oxidizing archaea (AOA)

2005: novel *amoA* genes in Archaea clones from German soil

Screening of a 1215 Mb soil metagenomic library





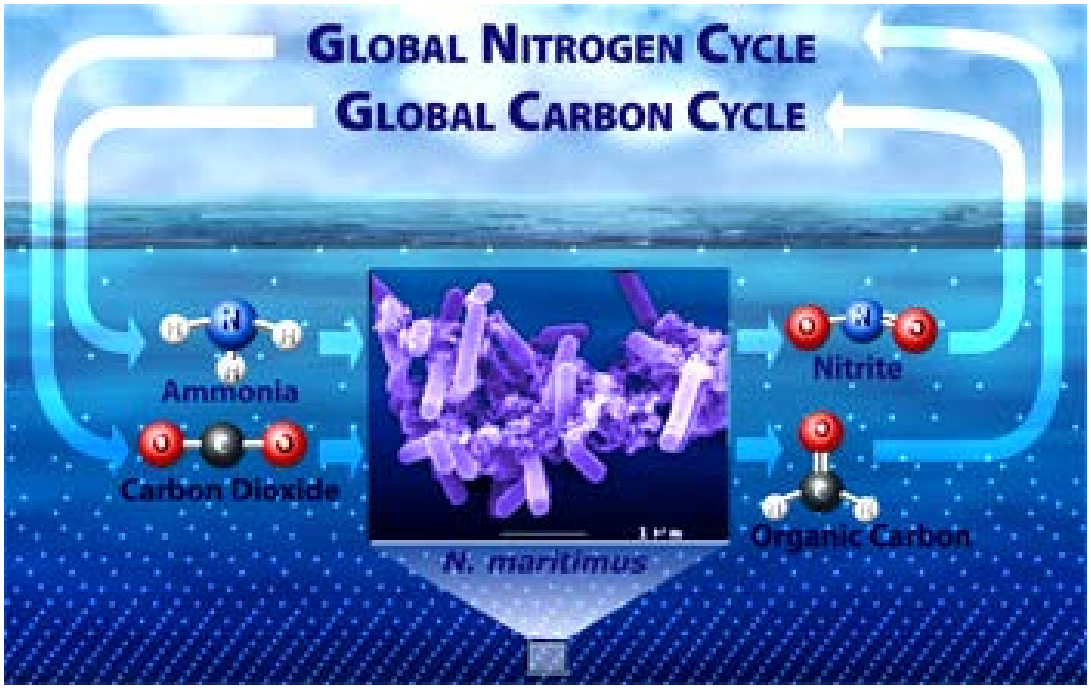


Treusch *et al*. 2005
Env Microbiol
7, 1985-1995

Archaea in nitrification

....

2005: First isolate, first observed nitrification in the marine archaea *Nitrosopumilus maritimus*



NH_3 oxidation and CO_2 fixation

Archaea in nitrification

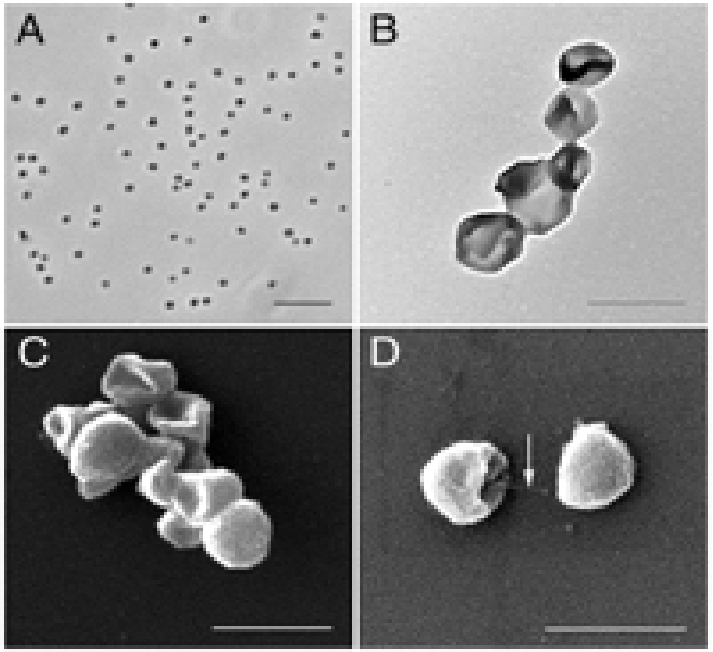
....

2005: Diversity of AOA in sea water and sediment

2006: AOA dominate over AOB in soils

2010: AOB active ammonia oxidizers in N rich soil, AOA in N poor soil

2011: First AOA soil isolate



PNAS

Ubiquity and diversity of ammonia-oxidizing ar… in water columns and sediments of the ocean

Christopher A. Francis*†, Kathryn J. Roberts‡, J. Michael Beman‡, Alyson E. Santoro§, and Brian B. Oakle…

Departments of *Geological and Environmental Sciences and ‡Civil and Environmental Engineering, Stanford University, Stanford, CA… and §Department of Microbiology, University of Washington, Seattle, WA 98195

Communicated by Pamela A. Matson, Stanford University, Stanford, CA, August 5, 2005 (received for review June 22, 2005)

Nitrification, the microbial oxidation of ammonia to nitrite and nitrate, occurs in a wide variety of environments and plays a central role in the global nitrogen cycle. Catalyzed by the enzyme ammonia monooxygenase, the ability to oxidize ammonia was previously thought to be restricted to a few groups within the β- and γ-Proteobacteria. However, recent metagenomic surveys have revealed the existence of unique ammonia monooxygenase subunit (amoA) genes derived from uncultivated mesophilic Crenarchaeota. Here, we report molecular evidence for the widespread presence of ammonia-oxidizing archaea in marine water columns and sediments. Using PCR primers that specifically target archaeal amoA, we find AOA in diverse areas of the ocean that are critical for the global N cycle, including the base of the euphotic zone, subeuphotic zone, and estuarine and coastal sediments. Diverse AOA communities are associated with each of these habitats, with overlap between water columns and sediments, but most AOA sequences are unique to sediments, whereas a small number of sequences are found in all locations, whereas a small number of sequences are found in all cosmopolitan in distribution. Considering the ubiquity of extremophilic archaea in the ocean, our results suggest that they may play a significant, but previously unrecognized, role in global nitrogen cycle.

Crenarchaeota | nitrification | ammonia monooxygenase

Nitrogen (N) is an essential nutrient in… biological productivity in most marine… global scale, biological N fixation is the larg… ocean, whereas anaerobic microbial process… N losses (1, 2). However, biological N fixat… losses are ultimately connected by nitrific… mediated, two-step conversion of ammoni… (NO_3^-) via nitrite (NO_2^-). As much as 4… through the ocean each year (1), and nearl… nitrified at least once (3). Although nitr… throughout the ocean, it plays a critical ro… by linking the decomposition of nitrogeno… loss via denitrification (microbial conversi… By removing a large percentage of anthr… from estuaries and continental shelf regi… the open ocean (4), coupled nitrification… vely isolates the marine N cycle fro… rrestrial N cycle (1). In the open ocean… curs in pelagic oxygen minimum zones… N losses have recently been attribute… n oxidation, or "anammox" (5). Duri… NH_4^+ occurs at the expense of NO… ototrophic NO_2^- reduction or aerobic… ep of nitrification. Nitrification is… ant process in OMZs and at ox… sediments, where the complex… denitrification, and anammox… nd large N losses to the atmo… ew phylogenetically restricte… own to perform either of the…

…/doi/10.1073/pnas.0506625102

Environmental Microbiology (2005) 7(12), 1985–1995

Novel genes for nitrite r… proteins indicate a role … crenarchaeota in nitroge…

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Summary

Mesophilic crenarchaeota are frequently foun… restrial and marine habitats worldwide, but… their considerable abundance the physiology… as yet uncultivated archaea has remained u… From a 1.2 Gb large-insert environmenta… library of a calcareous grassland soil,… genomic fragment was isolated with a ribosc… that shows its affiliation to group 1.1b of cr… ota repeatedly found in soils. The insert e… homologue of a copper-containing nitrite… with an unusual C-terminus that encoded … amicyanin-like electron transfer domain … two proteins related to subunits of ammo… oxygenases or particulate methane monoo… (AmoAB/PmoAB) respectively. Expressio… and the amoA-like gene was shown by re… scription polymerase chain reaction (PCR… in soil samples, the latter being found at hi… when the soil was incubated with amm… sured by quantitative PCR). Further varia… genes were amplified from soil sample… found in the environmental database fr… gasso Sea plankton. Taken together, … suggest that mesophilic terrestrial and m… chaeota might be capable of ammon… under aerobic and potentially also und… conditions.

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LETTERS

Archaea predominate among ammonia-oxidizing prokaryotes in soils

S. Leininger[1], T. Urich[1], M. Schloter[2], L. Schwark[3], J. Qi[4], G. W. Nicol[5], J. I. Prosser[5], …

…monia oxidizers. Our …e the most abundant …stems on Earth. …(AOB) of the β- and …en considered the most …ia oxidation[3,4]. These …raction of the micro- …ubunits of a potential …enzyme of AOB, on a …mal RNA operon of …aeota[8]. After addition …ription was induced[8]. …d a linked *amoC* gene …of the Sargasso Sea …ubunits in crenarch- …nia-oxidizing archaea …of a chemolithoauto-…

…bd (L–l) …lizer (L–n) …rgan, fert. (L–h)

…3,000 4,000 …OB

…Sandy (RUD)

1,200 …OB

…e copies in a sandy …all but one soil (L-h) …d constantly high (for …bars indicate standard …

…nment and Health, …ologne, Germany. …biological Sciences,

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LETTERS

Isolation of an autotrophic ammonia-oxidizing marine archaeon

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For years, microbiologists characterized the Archaea as obligate extremophiles that thrive in environments too harsh for other organisms. The limited physiological diversity among cultivated Archaea suggested that these organisms were metabolically constrained to a few environmental niches. For instance, all Crenarchaeota that are currently cultivated are sulphur-metabolizing thermophiles[1]. However, landmark studies using cultivation-independent methods uncovered vast numbers of Crenarchaeota in cold oxic ocean waters[2,3]. Subsequent molecular surveys demonstrated the ubiquity of these low-temperature Crenarchaeota in aquatic and terrestrial environments[4]. The numerical dominance of marine Crenarchaeota—estimated at 10^{28} cells in the world's oceans[3]—suggests that they have a major role in global biogeochemical cycles. Indeed, isotopic analyses of marine crenarchaeal lipids suggest that these planktonic Archaea fix inorganic carbon[5]. Here we report the isolation of a marine crenarchaeote that grows chemolithoautotrophically by aerobically oxidizing ammonia to nitrite—the first observation of nitrification in the Archaea. The autotrophic metabolism of this isolate, and its close phylogenetic relationship to environmental marine crenarchaeal sequences, suggests that nitrifying marine Crenarchaeota may be important to global carbon and nitrogen cycles.

Since their discovery by Fuhrman *et al.* and DeLong over a decade ago[2,3], marine Crenarchaeota are now recognized to be a dominant fraction of bacterioplankton in the ocean. These microorganisms can account for up to 40% of the bacterioplankton in deep ocean waters[3]. Although there are no known low-temperature Crenarchaeota in culture, compound-specific $\Delta^{14}C$ analysis of lipid biomarkers[6] and studies of ^{13}C-bicarbonate tracer uptake by natural populations[7] have suggested autotrophy. However, another study of natural populations demonstrated the uptake of tracer levels of tritiated amino acids, suggesting some use of fixed carbon[8]. We expect that the availability of a representative organism in pure culture will facilitate studies of their physiology and evolutionary origin, and help in understanding their contribution to oceanic biogeochemical cycles.

Known nitrifying bacteria fall into two distinct physiological groups: those that oxidize ammonia to nitrite, and others that oxidize nitrite to nitrate[9]. None has been shown to oxidize ammonia completely to nitrate. Existing genera of ammonia-oxidizing bacteria (AOB) fall within the Betaproteobacteria and the Gammaproteobacteria (ref. 9). Molecular studies of AOB in nitrifying systems, including aquaria[10], have been limited to these two phylogenetic groups[9]. We first suspected an involvement of Archaea in nitrification after we completed several cultivation-independent ribosomal RNA gene surveys of nitrifying environments. We detected sequences affiliated with the marine group 1 Crenarchaeota in nitrifying dilution cultures developed from Plum Island Sound (Massachusetts) estuary sediment, in nitrifying filtration systems at the Shedd Aquarium (Chicago, Illinois), and in gravel from a marine tropical fish tank at the Seattle Aquarium (Seattle, Washington).

Further evidence for archaeal nitrifiers resulted from ammonia-oxidizing cultures highly enriched in marine group 1 Crenarchaeota. Filtered aquarium water (0.2-μm polyethersulphone membrane; Nalgene) supplemented with 1 mM ammonium chloride was inoculated with gravel from a tropical marine tank at the Seattle Aquarium. Cultures enriched for Crenarchaeota were incubated at 21–23 °C in the dark. Repeated serial transfers of 10% of the culture volume into fresh aquarium-water medium resulted in an enrichment comprised of approximately 90% Crenarchaeota and 10% organisms affiliated with the bacterial domain after six months (data not shown). Characterization of this highly enriched culture revealed that oxidation rates of ammonia to nitrite corresponded with increasing abundance of Crenarchaeota (measured by quantitative polymerase chain reaction (PCR); Supplementary Information) indicating nitrification (data not shown).

After initial enrichment, the Crenarchaeota were isolated in a defined medium (see Methods) containing bicarbonate and ammonia as the sole carbon and energy sources, suggesting autotrophy. A pure culture of Crenarchaeota (designated SCM1) was recovered after three serial end-point dilutions in this medium, facilitated by the addition of streptomycin and filtration of the inoculum through a 0.45-μm HT Tuffryn membrane syringe filter (Pall). The purity of SCM1 was confirmed by quantitative PCR and fluorescent *in situ* hybridization (FISH), and supported by a failure to recover bacterial 16S rRNA genes by PCR amplification or to promote the growth of heterotrophic bacteria by the addition of yeast extract and peptone to the defined culture medium (data not shown). PCR amplification of nearly full-length 16S rRNA genes from SCM1 identified only crenarchaeal sequences. The clonal structure of SCM1 was confirmed by comparing the sequences of PCR-amplified fragments of 1,650 base pairs (bp) in length and containing most of the 16S rRNA, the complete 16S–23S internal transcribed spacer, and a small portion of the 23S rRNA gene (Supplementary Information).

Comparative sequence analysis of 16S rRNA genes revealed a high level of sequence identity (>98%) between SCM1 and marine group 1 Crenarchaeota sequences recovered from the North Atlantic, the Red Sea, the Antarctic and hydrothermal vents (Fig. 1). Phylogenetic analysis indicates that all marine group 1 Crenarchaeota—including SCM1, crenarchaeal sequences from the Sargasso Sea[11] and *Cenarchaeum symbiosum* (an uncultured marine sponge symbiont)[12]—form a monophyletic clade sharing >94% rRNA sequence identity (Fig. 1).

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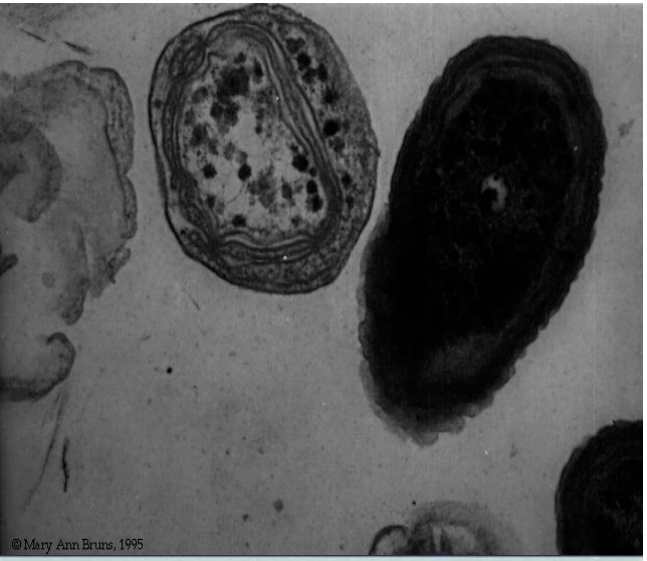
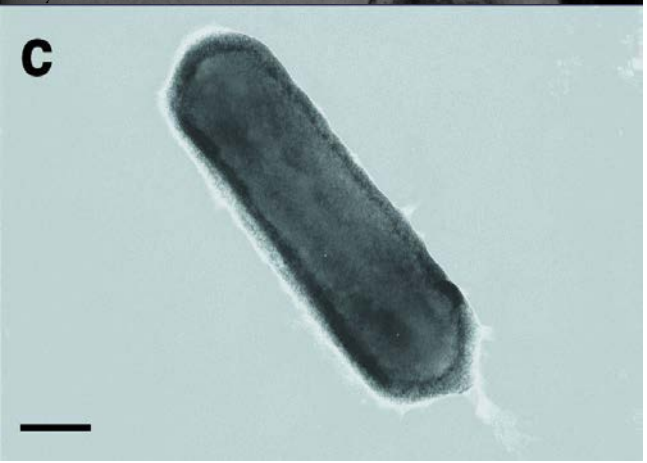
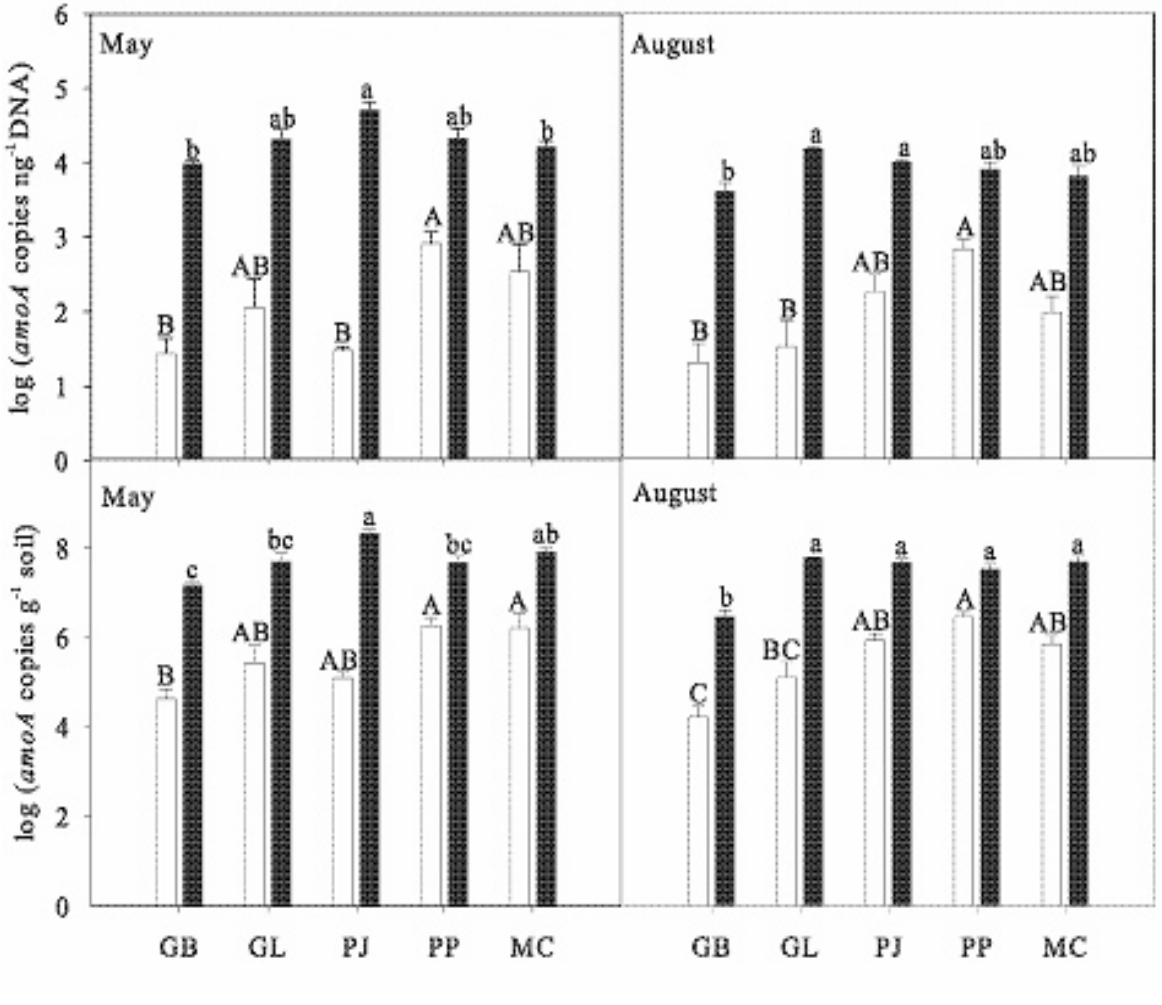
*These authors contributed equally to this work.

Archaea predominate among ammonia-oxidizing prokaryotes in soils







AOA to AOB ratio in soil

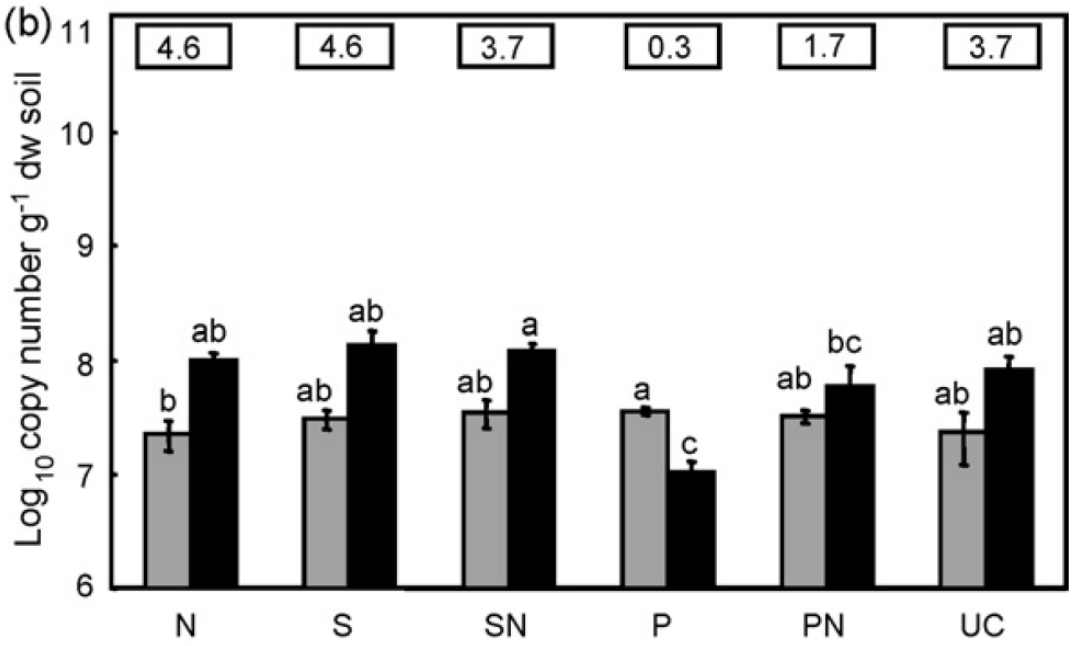
By soil depth:

0-10 cm	55:1
20-30 cm	170:1
40-50 cm	1125:1

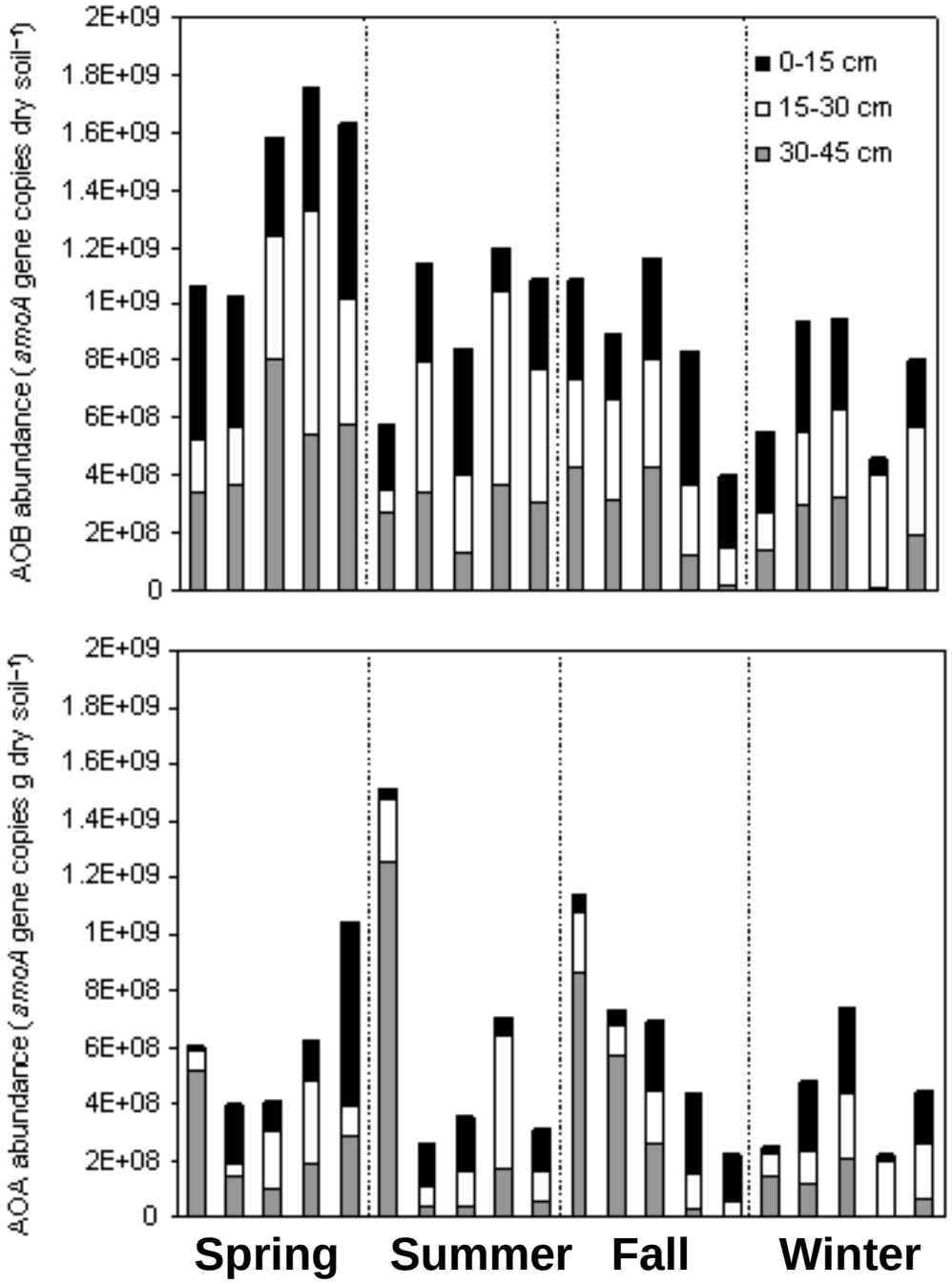
The presence of a gene does not prove function.

N turnover rates?

- Archaeal ammonia oxidizers typically dominate in soils, but not always.
- Why? Does it matter?

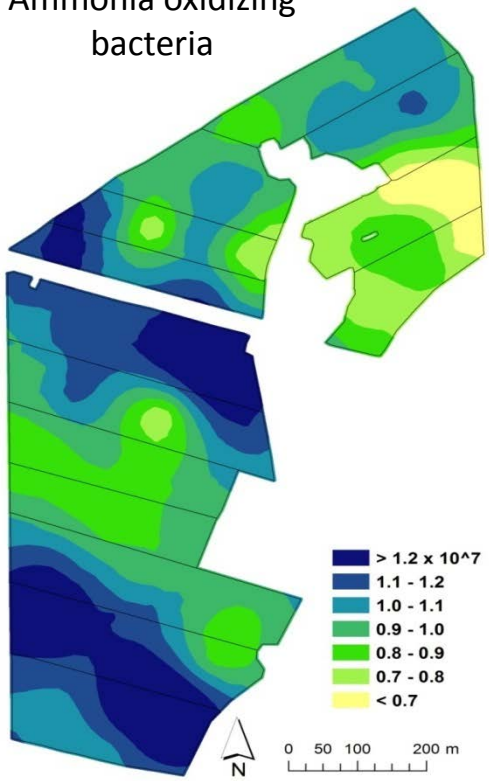


(Wessén et al*, 2010 *Appl Soil Ecol)


AOB abundance (amoA gene copies dry soil-1)
2E+09
1.8E+09
1.6E+09
1.4E+09
1.2E+09
1E+09
8E+08
6E+08
4E+08
2E+08
0
0-15 cm
15-30 cm
30-45 cm
AOA abundance (amoA gene copies g dry soil-1)
2E+09
1.8E+09
1.6E+09
1.4E+09
1.2E+09
1E+09
8E+08
6E+08
4E+08
2E+08
0
Spring
Summer
Fall
Winter

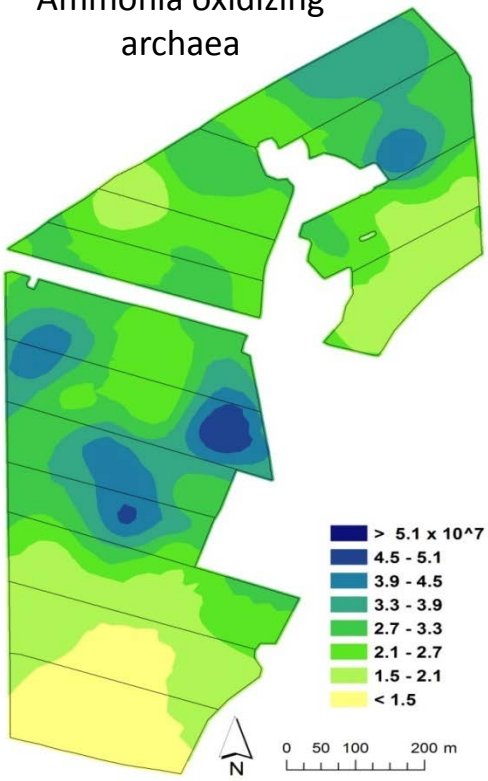
Abundance (quantative PCR *amoA*)

Ammonia oxidizing bacteria



+ Tot-C
+ Tot-N

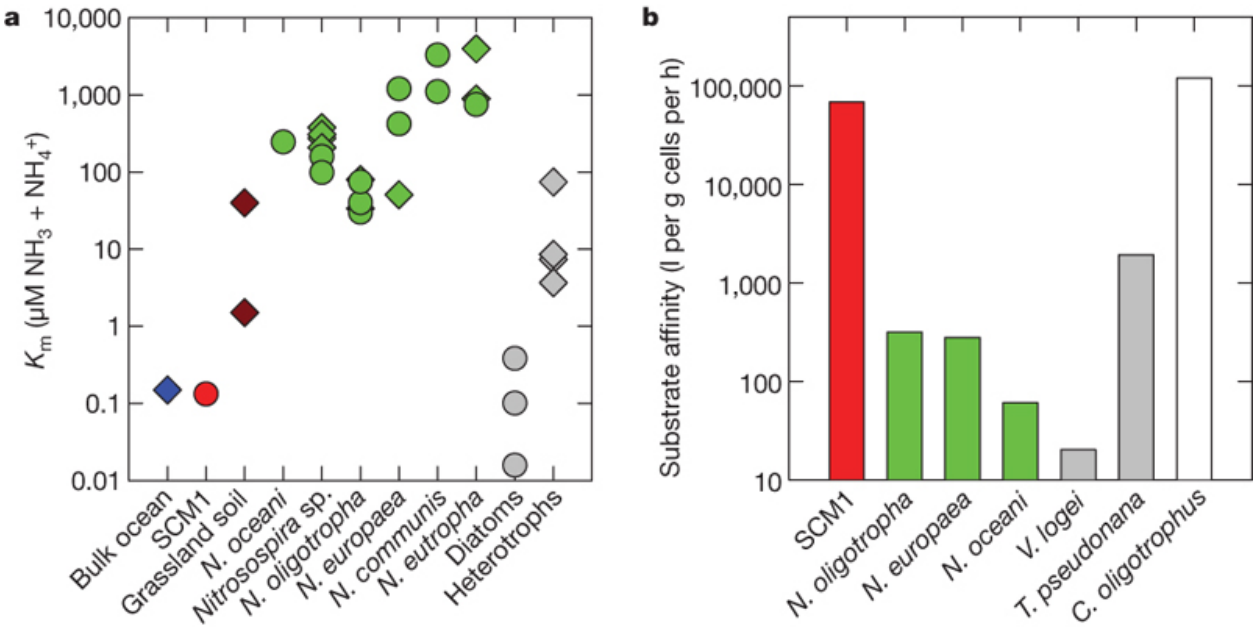
Ammonia oxidizing archaea



- pH
- Clay

(Wessén et al, 2011 ISME J)

High-affinity ammonia oxidation by AOA dominates in oligotrophic environments.



a, K_m of AOA (red), AOB strains (green), *in situ* nitrification in ocean water (blue) and soils (brown), as well as the lowest K_m for ammonium assimilation of diatoms and heterotrophic bacteria (grey). K_m values are given for activity measurements (circles) and growth (diamonds).
b, Specific affinitiy (a^0) of AOA (red), AOB(green), as well as the highest values for ammonium-assimilating diatoms, and heterotrophic bacteria (grey).

(Martens-Habbena *et al. Nature*, 2009)