

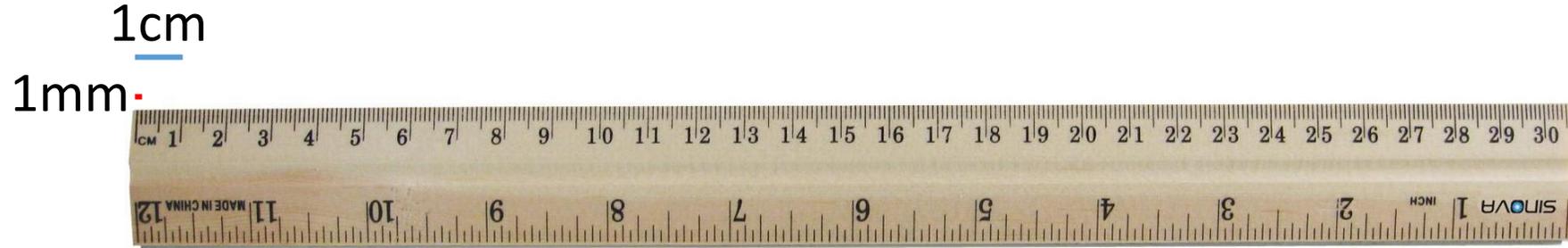
Exploring Microbes in the Sea

Alma Parada
Postdoctoral Scholar
Stanford University

Cruising the ocean to get us some microbes



How tiny is a microbe?

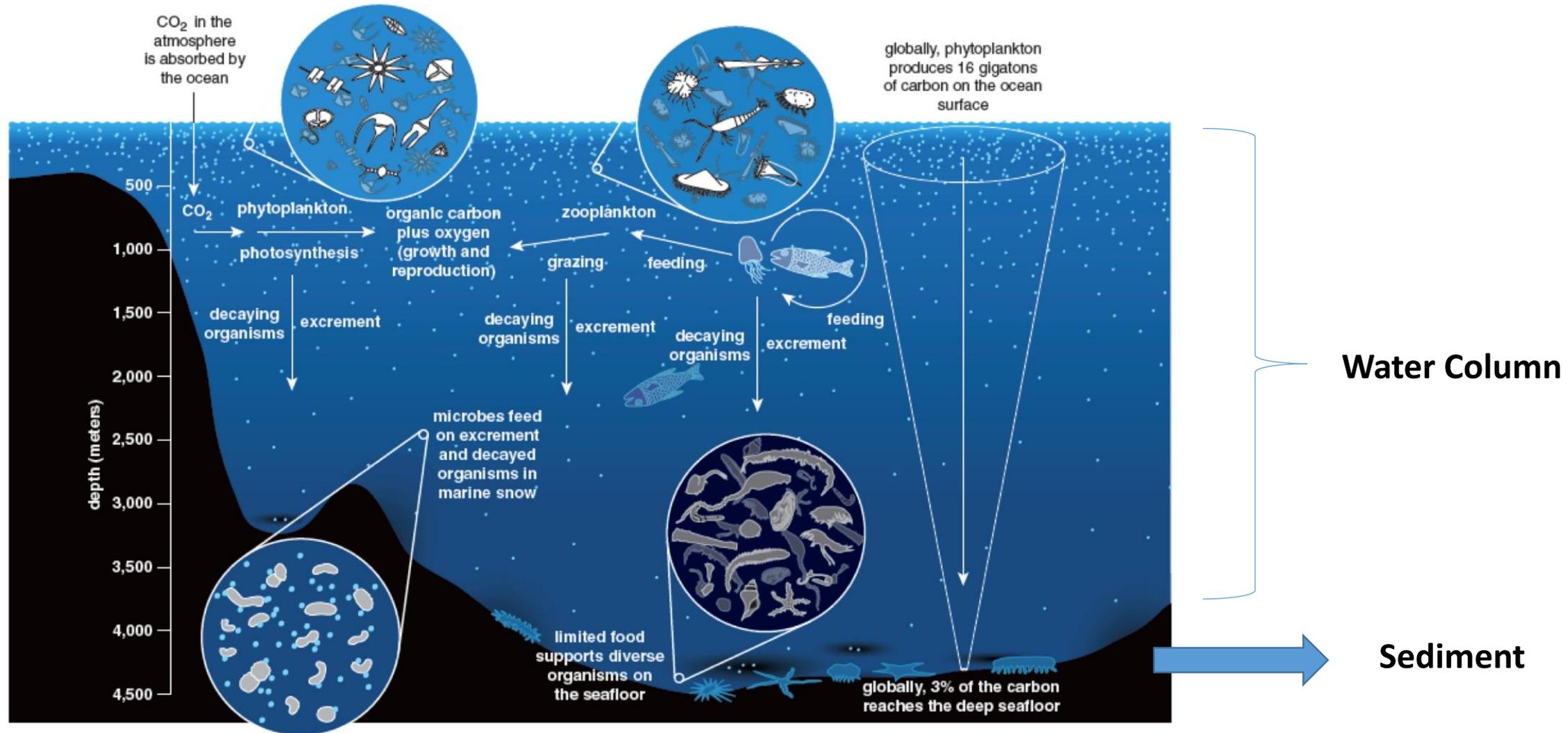


1mm =
1/10 cm

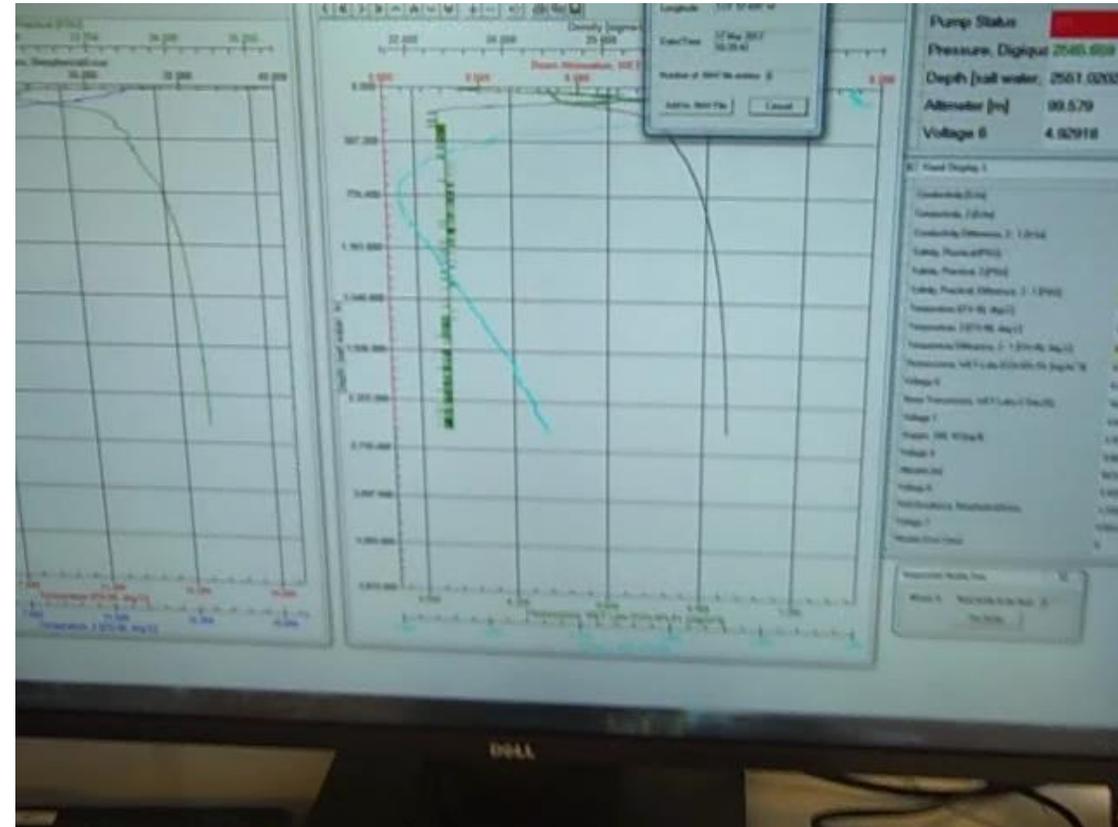
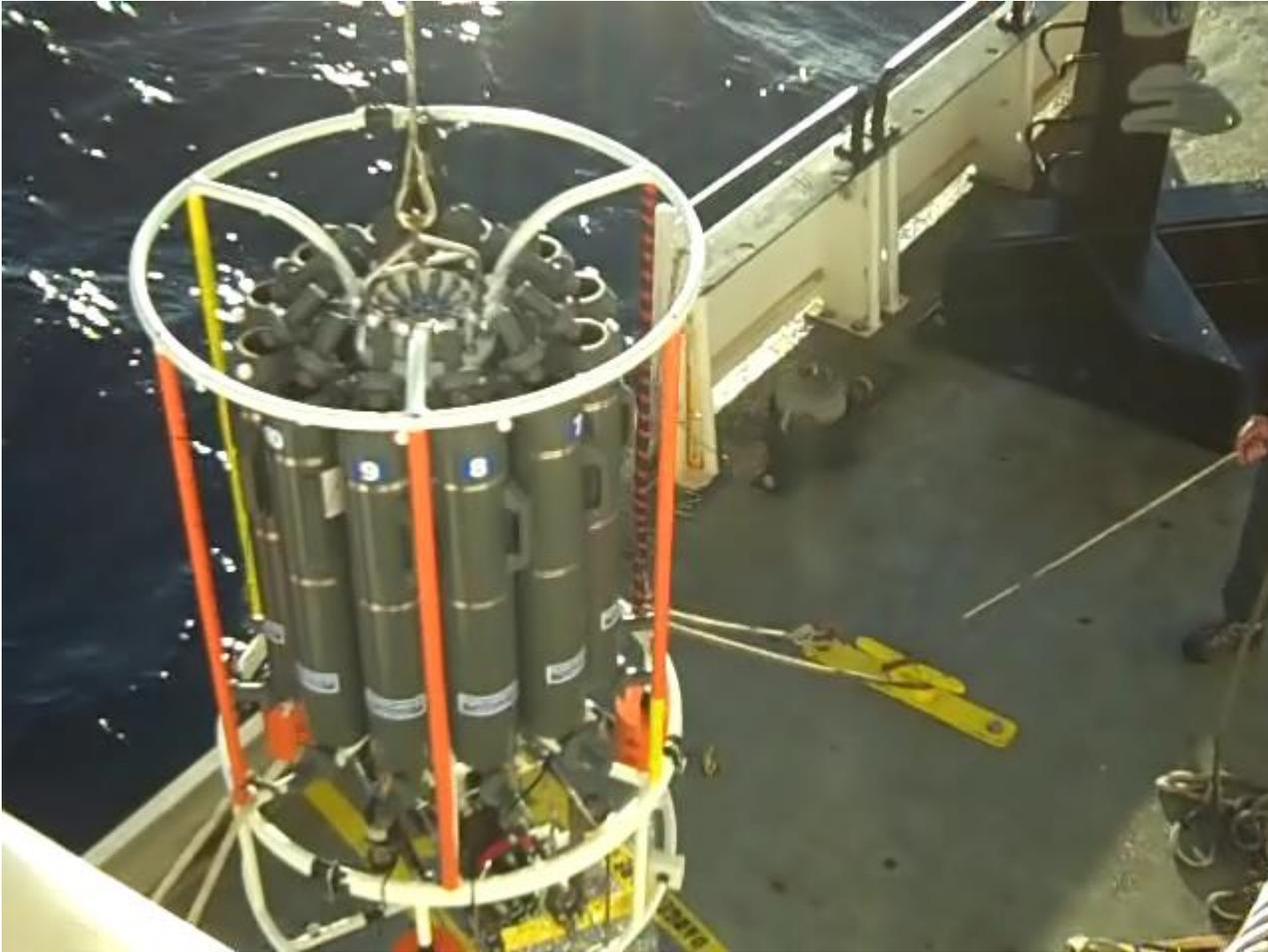
1μm =
1/10,000 cm
= 0.000001 meter

	Size category	Size range (μm)	Microbial groups	
SIZE 	Femtoplankton	0.01–0.2	Viruses	 ABUNDANCE
	Picoplankton	0.2–2	<i>Bacteria</i> ^a , <i>Archaea</i> , some flagellates	
	Nanoplankton	2–20	Flagellates, diatoms, dinoflagellates	
	Microplankton	20–200	Ciliates, diatoms, dinoflagellates, other algae	

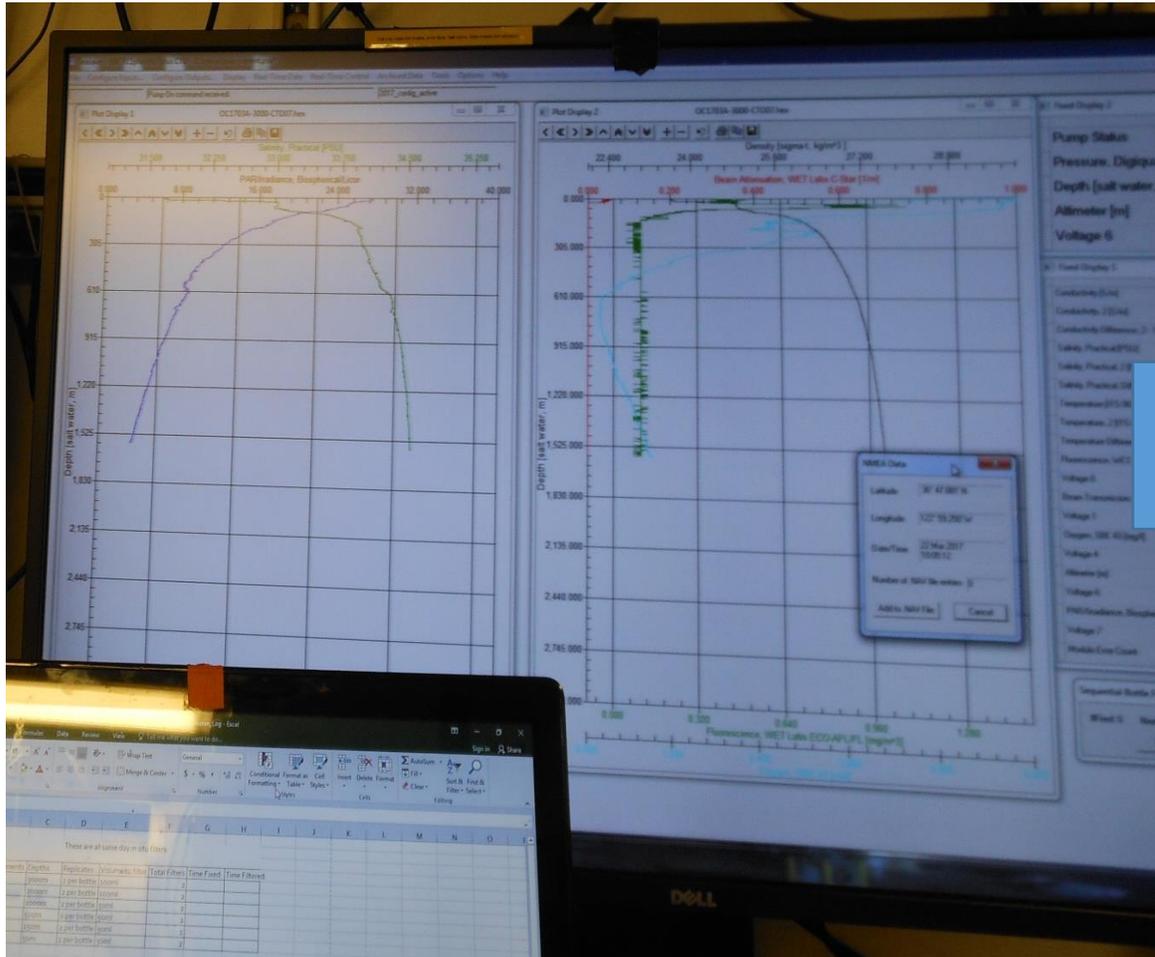
Where do the microbes live?



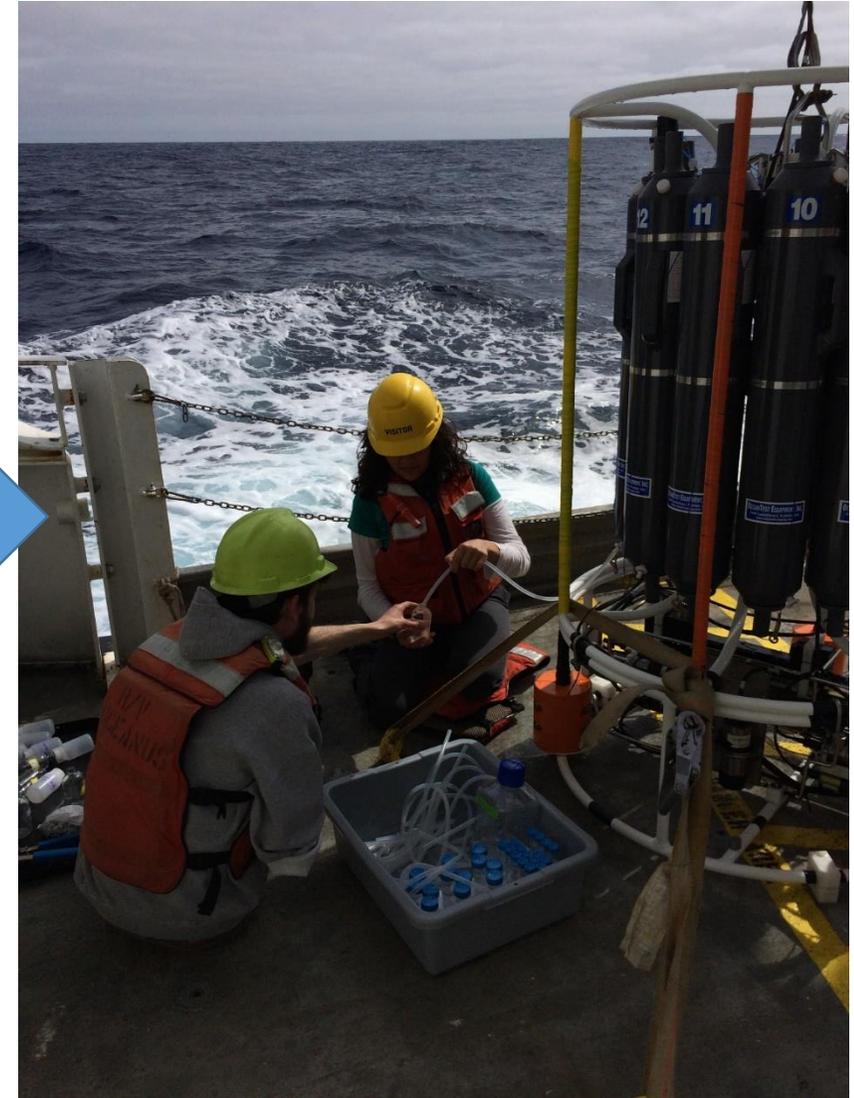
Getting microbes from the water using Niskin bottles!



Getting Water Column Microbes



Subsample
different depths



Use Pressure to push water through filters



Microbial Community Directed Research Questions

Now that we've captured our microbes on filters.....

- What is the microbial community composition?
 - Who is there, when and why?
- How dynamic is the microbial community?
 - Does the community change over time or in response to environmental changes?
- What are the interactions that shape the microbial community?
 - How do microbes affect each other? How does the environment affect microbes?

Methods to Evaluate a Microbial Community

- One important method: **DNA!**

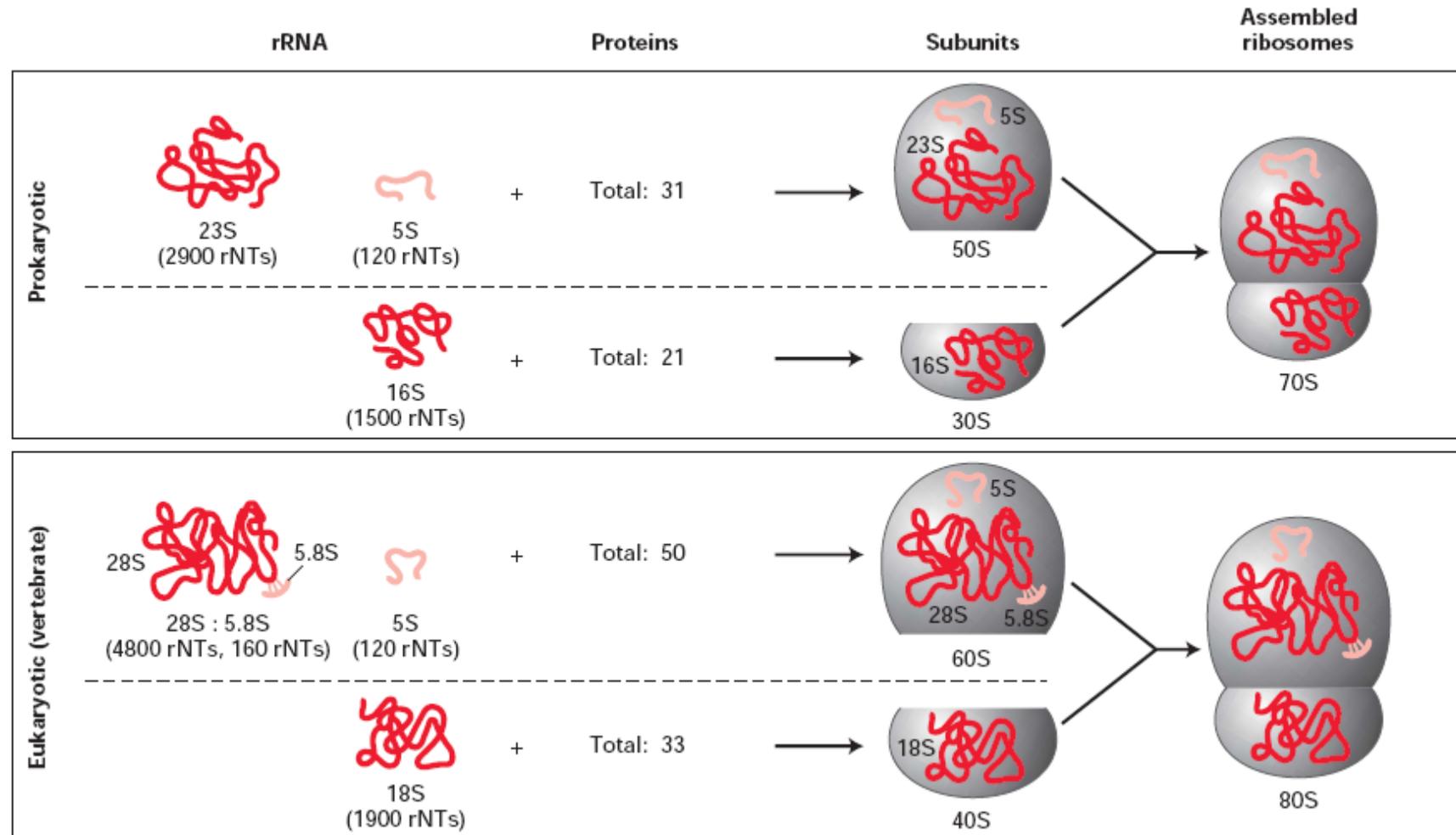
- An organism's DNA contains all of the **genes (information)** it needs to live

- These genes include those that code for **ribosomes!**

- **This is where proteins are built!**

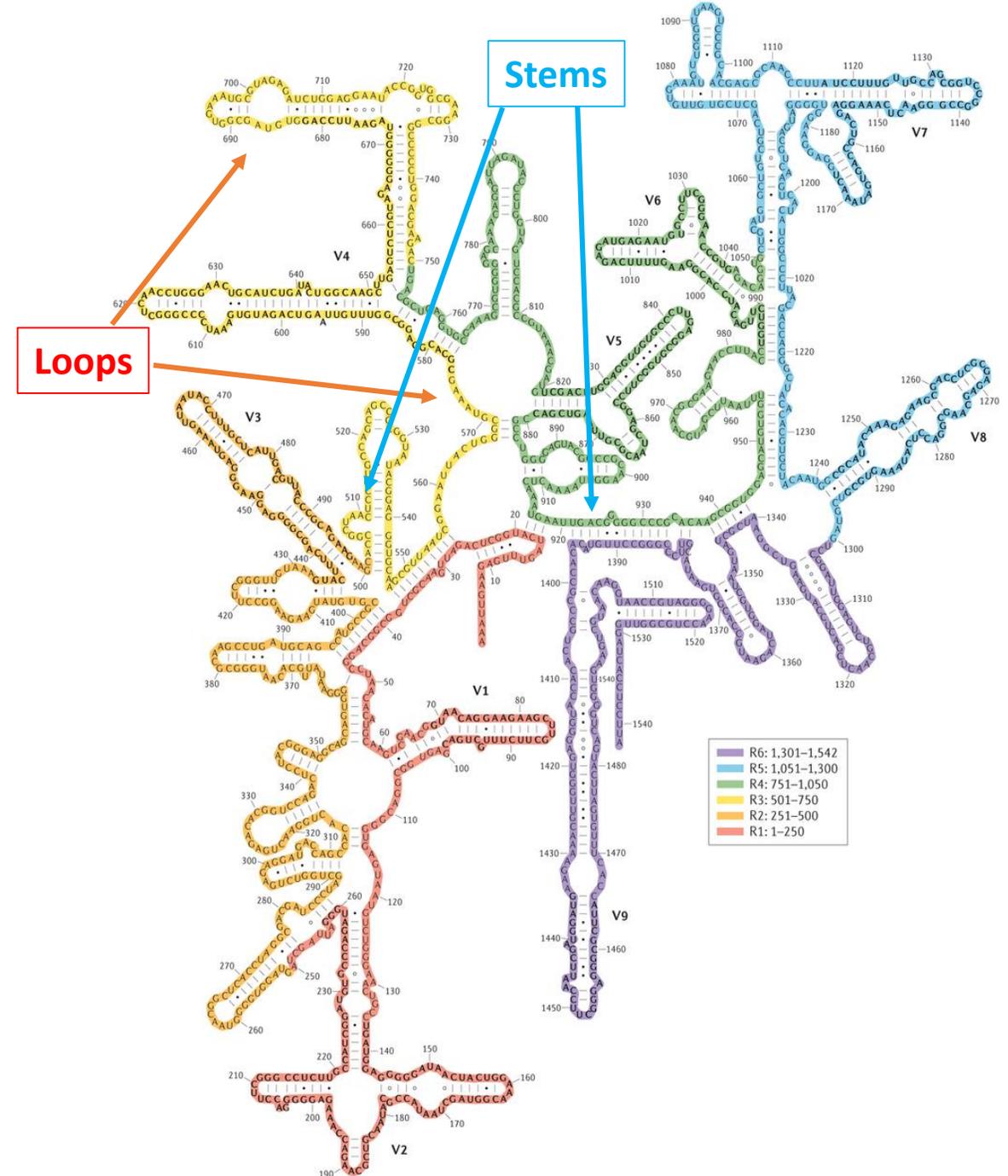
- 2-unit structure made of ribosomal RNA (rRNA) and Proteins

- The rRNA occurs in 3 chunks- one of which is the **16S**

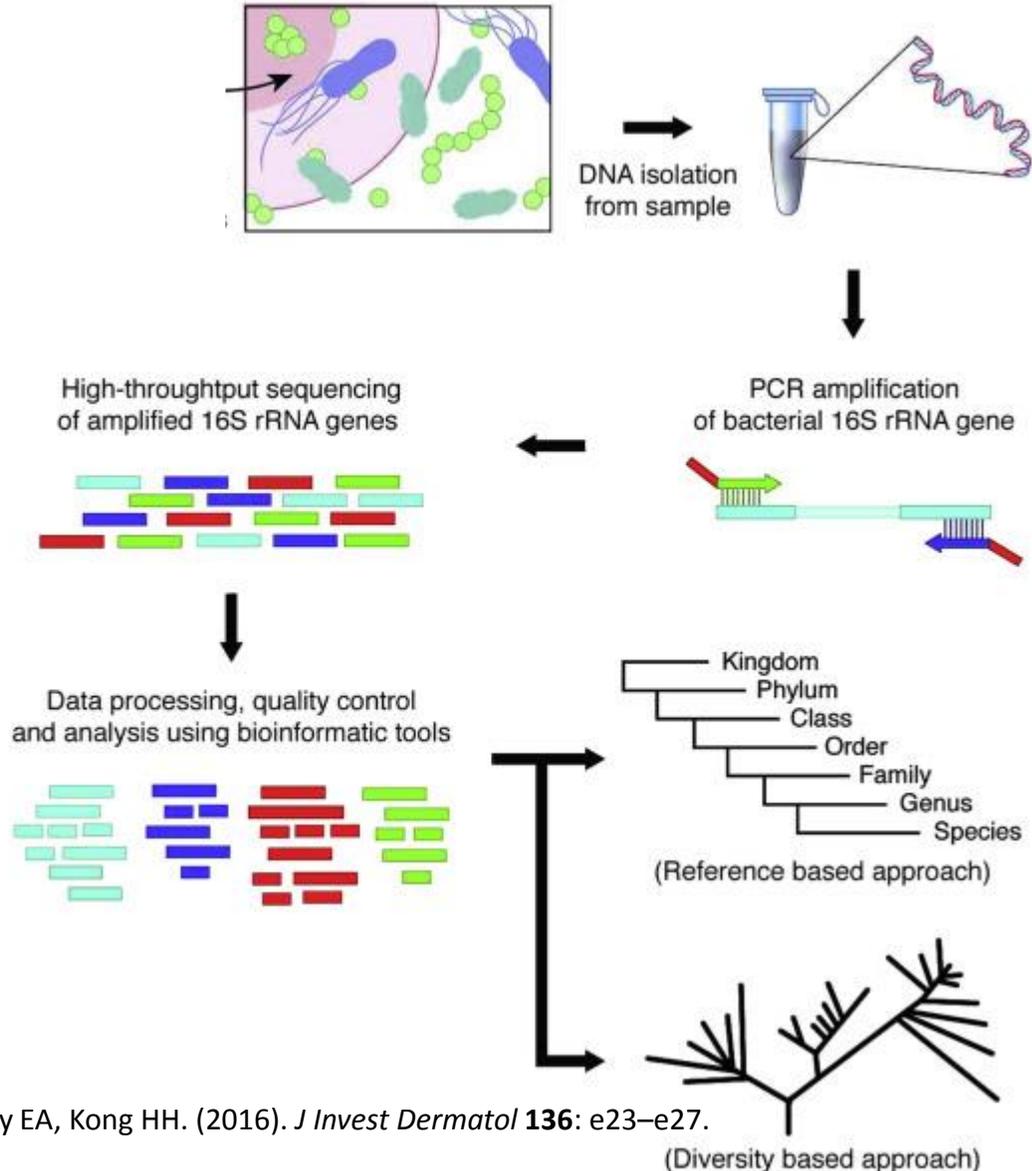


16S rRNA Molecule

- Loops are sequences that can mutate more easily
 - Makes them **variable** between related groups of organisms
 - i.e. phylogenetic group
 - Therefore the **sequence of 16S rDNA** of these hypervariable regions allow us to **distinguish phylogenetic groups** from each other
- Stems are sequence regions less prone to mutation
 - Makes them **conserved** between groups of organisms
 - These are candidate regions for Polymerase Chain Reaction (**PCR primers**) to target many phylogenetic groups



Sequencing the 16S rDNA



• PCR Amplification

- Extract DNA from environment
 - This contains the genomes from all microorganisms in the sample
- Uses primers to target 16S rDNA regions of each genome and make lots of copies (amplicons)

• Sequencing

- Can use different platforms to determine the **sequence** of all those 16S rDNA amplicons

• Bioinformatics

- Use the **amplicon sequences** to determine **identify of the microbes**
- Use information added to the primers to determine which sequences belong to different samples

Getting from 16S rDNA sequences to microbe identity

- Because PCR and sequencing can introduce errors in the 16S rDNA sequences we need methods to distinguish between errors and real sequence (genetic) diversity
- 16S rDNA sequences are commonly clustered by sequence similarity into
 - Operational Taxonomic Units (OTUs)
 - The term “operational” comes from the idea, that *how* that taxonomic unit is defined can be flexible
 - The term OTU is most commonly used in reference to groups of similar 16S rRNA gene (rDNA) sequences
 - Typically an OTU is considered to define a cluster of sequences that originate from a single type of microbe
 - Of course this is not always true, but new methods are developed all the time to get us to approach the coveted OTU=Species or Strain

How do we make an OTU?: Sequence Clustering

- Making Operational Taxonomic Units
 - Common strategies:
 - Reference Based: compare sequences to reference set, discard all other sequences
 - De novo based: use the sequences to determine how the clusters are formed
 - These common strategies require user defined similarity thresholds
 - Essentially, you choose an algorithm that will cluster the sequences into OTUs based on a sequence similarity cutoff that you have chosen
 - Most common similarity is 97% OTUs, but since sequences that are 97% similar can group together organisms that are quite different, it is not uncommon to require a greater similarity threshold to define an OTU (ie. 99% OTUs or Unique Sequences)

You've got OTUs... Now what?

- After you make OTUs:
 - you want to know how abundant an OTU is in each sample
- An OTU table tells you how many sequences belong to each OTU in each sample

	Sample 1	Sample 2	Sample 3	Sample 4
OTU1	1	200	250	5
OTU2	5	8	3	10
OTU3	100	2	2	80
OTU4	3	200	450	1000
OTU5	500	1500	600	1000

Making an OTU table usable

- To allow us to compare the OTU abundance between samples:
- One method is to:
 - Rescale sequence counts to relative abundance
 - Calculate Percent Relative Abundance for each OTU in each sample

OTU % Relative Abundance per sample

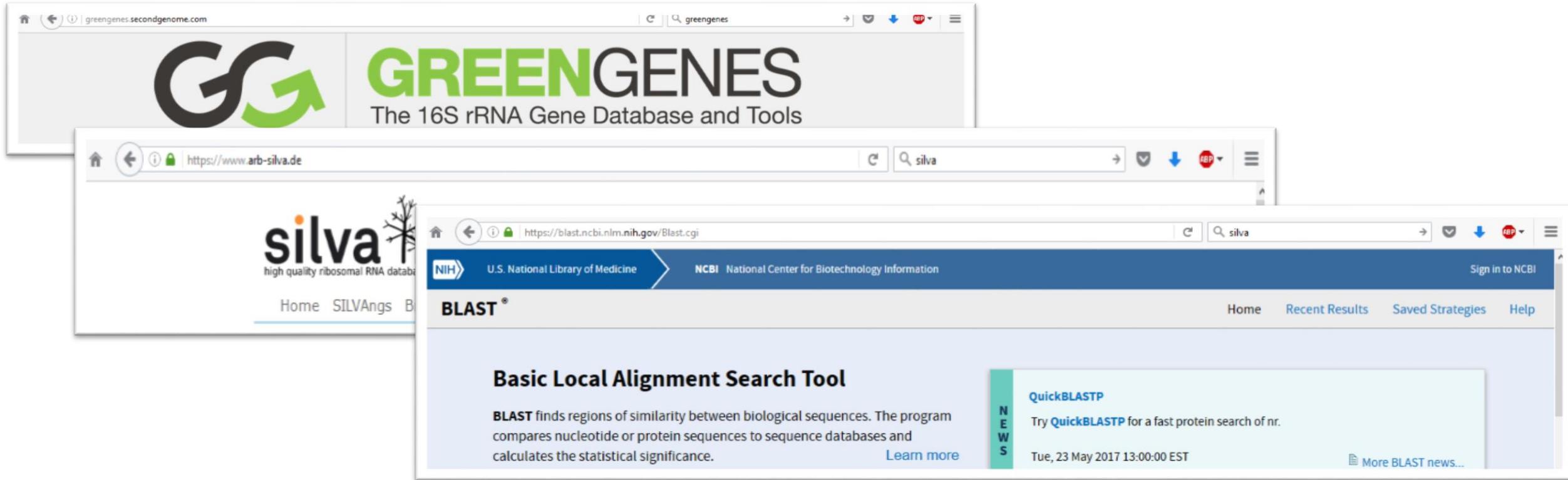
$$= 100 \left(\frac{\text{OTU Sequence Count in Sample}}{\text{All Sequences in Sample}} \right)$$

- But what is the **identity** of each OTU?

OTU Sequences per Sample				
	Sample 1	Sample 2	Sample 3	Sample 4
OTU1	1	200	250	5
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OTU3	100	2	2	80
OTU4	3	200	450	1000
OTU5	500	1500	600	1000

OTU % Relative Abundance per Sample				
	Sample 1	Sample 2	Sample 3	Sample 4
OTU1	0.16	10.47	19.16	0.24
OTU2	0.82	0.42	0.23	0.48
OTU3	16.42	0.10	0.15	3.82
OTU4	0.49	10.47	34.48	47.73
OTU5	82.10	78.53	45.98	47.73

Using existing 16S rDNA Databases



- Compare **representative sequences** from each OTU to a database
 - This tells us what phylogenetic group each OTU is most similar to
 - This allows us to **assign taxonomy** to each OTU

OTU Table becomes a window into the Microbial Community

- Now we can get back to our research questions!

OTU % Relative Abundance per Sample				
	Sample 1	Sample 2	Sample 3	Sample 4
Nitrosopumilus maritimus	0.16	10.47	19.16	0.24
Prochlorococcus	0.82	0.42	0.23	0.48
Euryarchaea	16.42	0.10	0.15	3.82
SAR11	0.49	10.47	34.48	47.73
Unknown	82.10	78.53	45.98	47.73

My Research Questions

- How dynamic are the two most numerically abundant archaeal communities in the marine water column?
 - What is the distribution of the Thaumarchaea Marine Group I (MGI) and Euryarchaeal Marine Group II (MGII) communities across the water column?
 - **Hypothesis:** Based on their metabolisms and lifestyles the MGI will be more abundant with depth, while the MGII will be more abundant in the surface ocean.
 - Does their community composition change over time?
 - **Hypothesis:** The community structure of each group will change in response to temporal changes in the availability of potential substrates for growth or other environmental conditions that affect their growth rates and abundances.
 - How do the MGI and MGII fit into the rest of the microbial community?
 - **Hypothesis:** Members of the MGI and MGII will correlate to other microbes that have synergistic activities, while competition with other microbes will negatively impact the abundances of these groups.

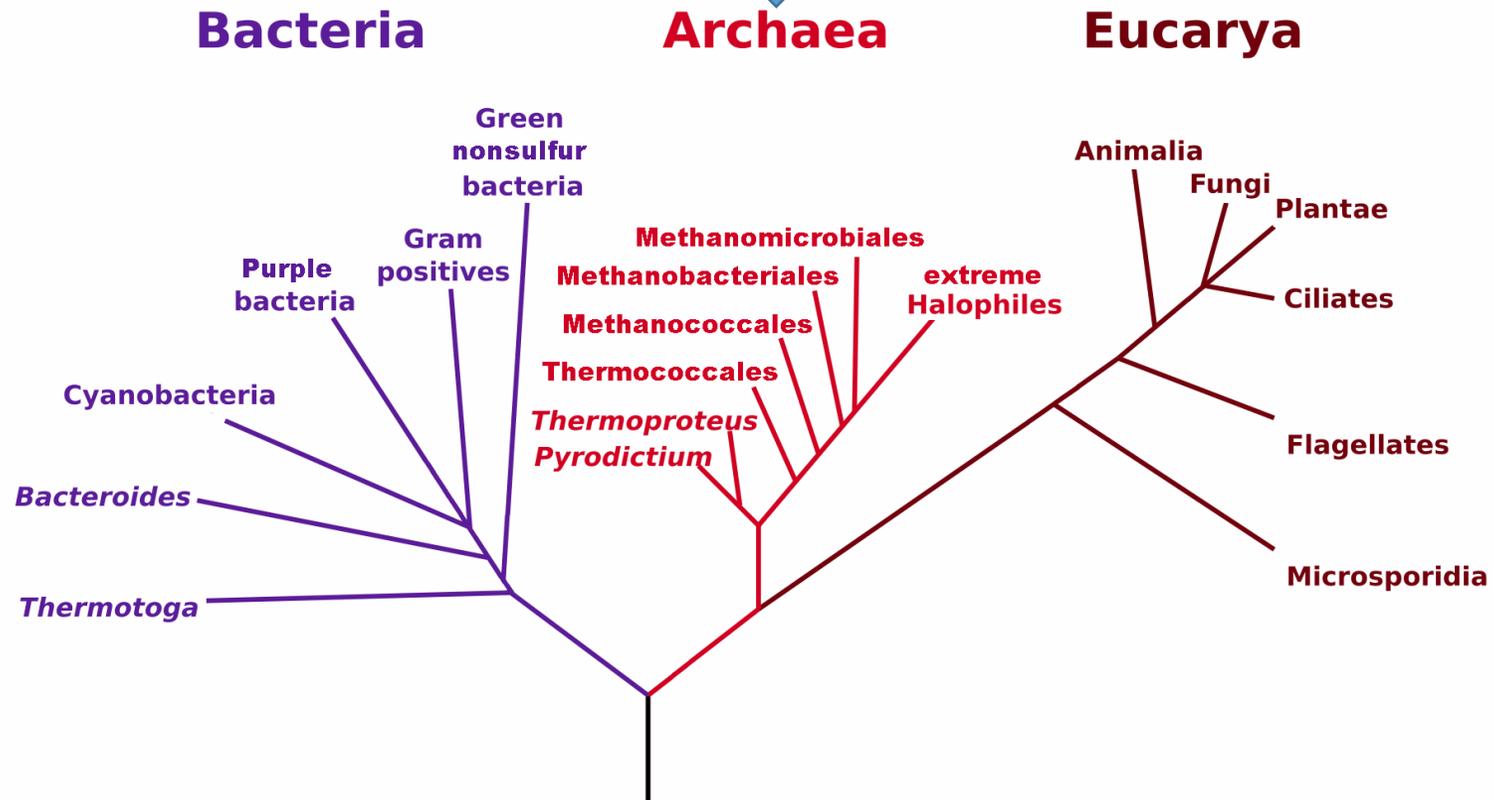
Thaumarchaea MGI and Euryarchaea MGII

- Let's take a quick step back
- Who are the MGI and MGII and what do we know about them?

Tree of life

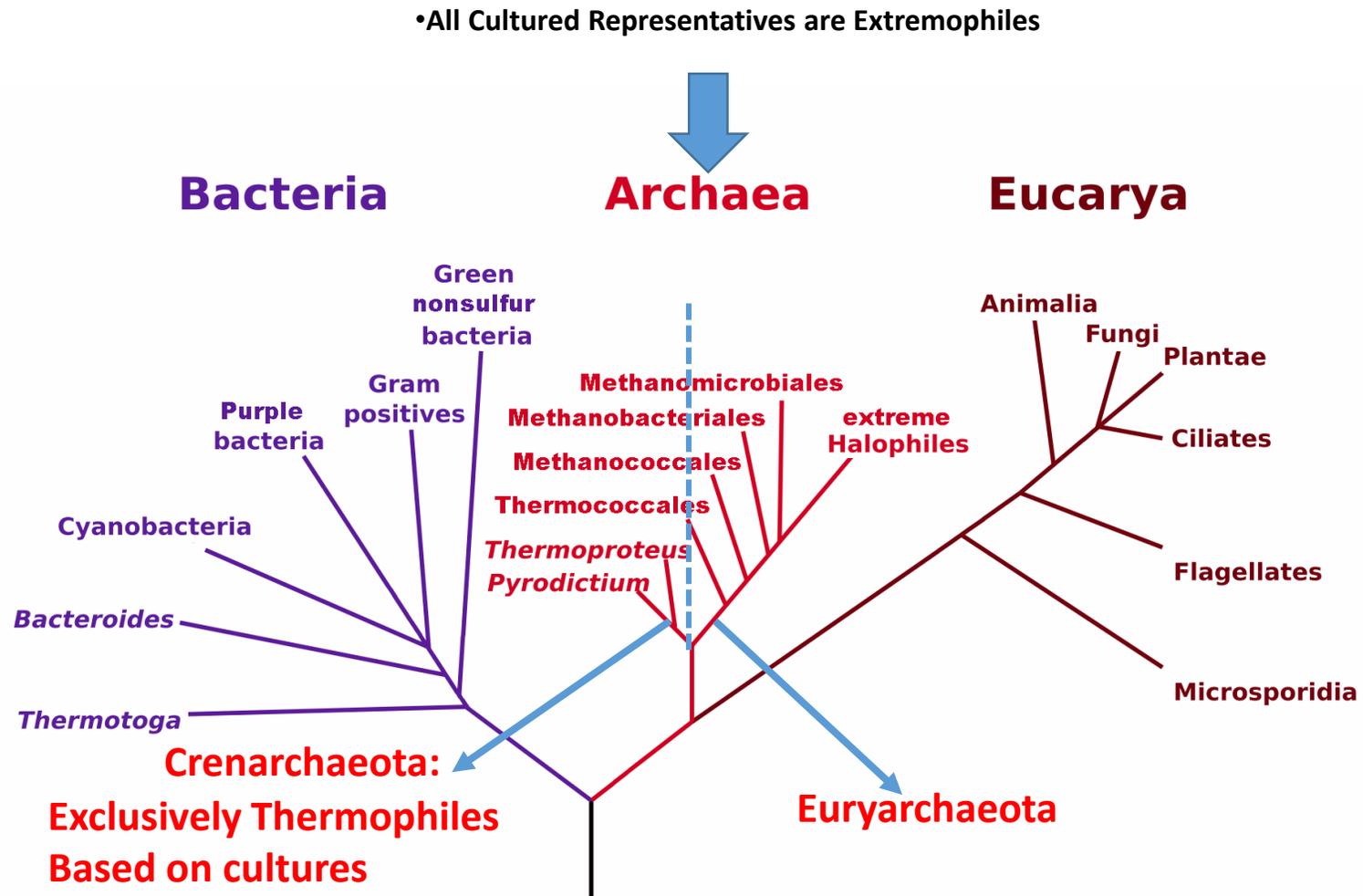
- A 3-domain system formally proposed by Carl Woese and colleagues 1990 (PNAS)

• Concept developed in 1977



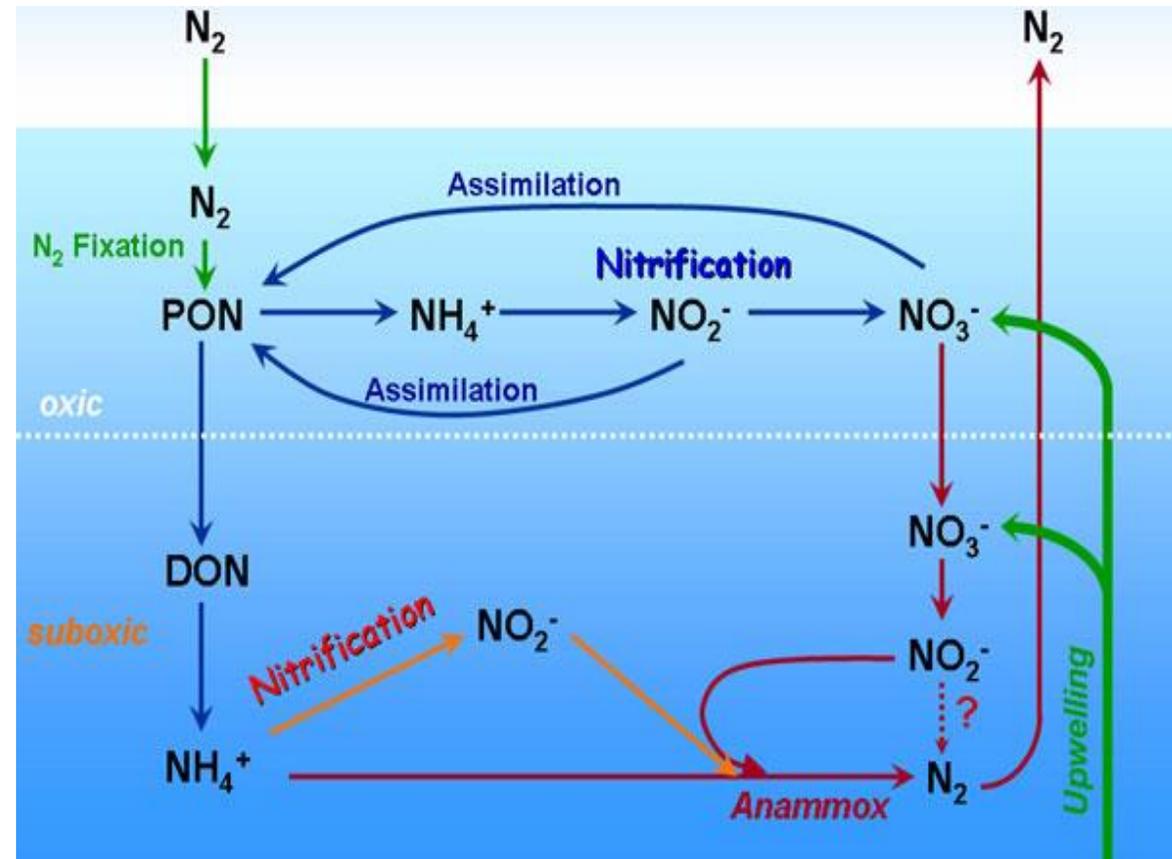
Tree of life

- A 3-domain system proposed by Carl Woese and colleagues 1990 through use of the 16S rRNA gene



Thaumarchaea MGI found throughout the ocean! So not just an extremophile!

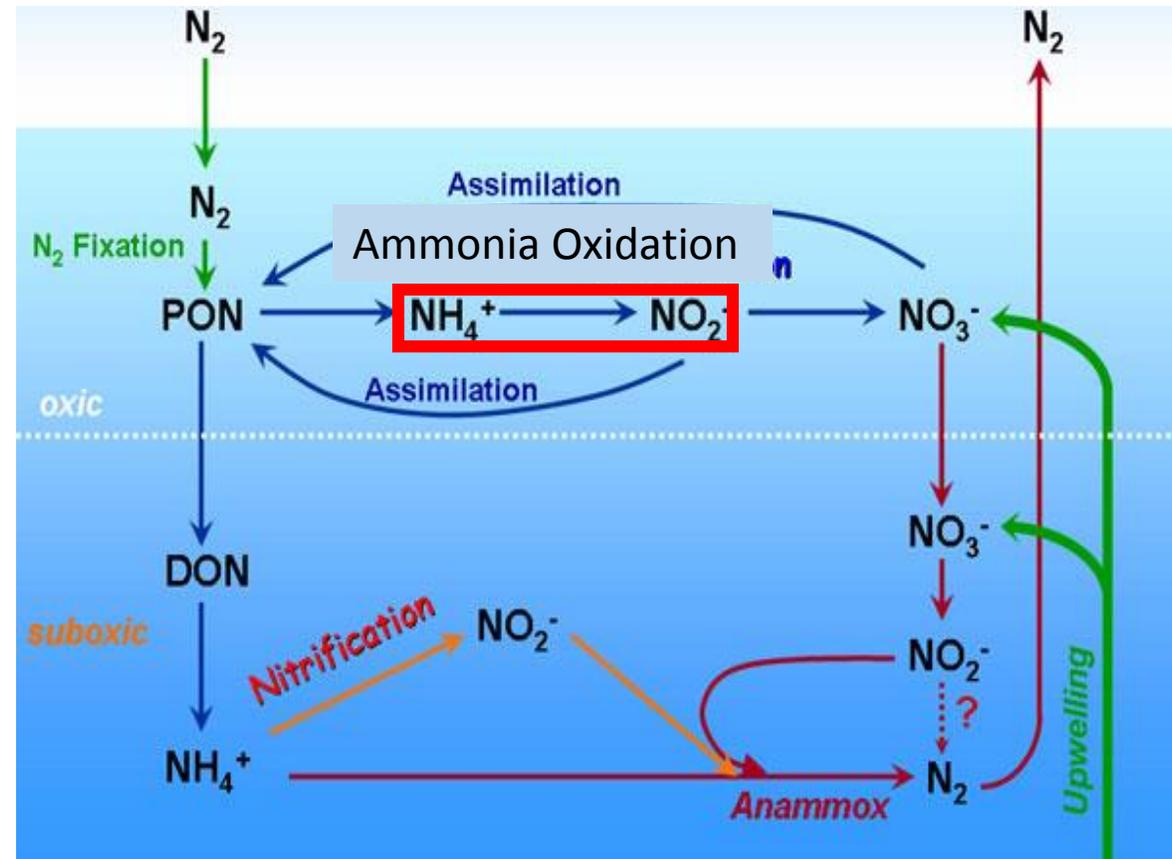
- Not only not an extremophile, evidence showed many MGI are autotrophs that have an important role in the nitrogen cycle
 - (e.g. Karner et al. 2001, Pearson et al. 2001, Ouverney et al. 2000, Venter et al 2004)



Lam et al (2007). PNAS

Thaumarchaea MGI found throughout the ocean! So not just an extremophile!

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 - (e.g. Karner et al. 2001, Pearson et al. 2001, Ouverney et al. 2000, Venter et al 2004)
- *Nitrosopumilus maritimus*: isolate that performs autotrophic ammonia oxidation
 - Könneke et al (2005)



Lam et al (2007). PNAS

MGI life is more unclear

- Their genomes indicate heterotrophic lifestyles
 - e.g. Iverson *et al.* 2012, Orsi *et al.* 2015, Martin-Cuadrado *et al.* 2015
- Incubation experiments show incorporation of organic carbon
 - Orsi *et al.* 2015, 2016
- However, all of our genetic information comes from organisms living in the photic zone

ORIGINAL ARTICLE

Marine archaeal dynamics and interactions with the microbial community over 5 years from surface to seafloor

Alma E Parada and Jed A Fuhrman

Department of Biological Sciences, University of Southern California, Los Angeles, CA, USA

Samples & Methods to study the MGI and MGII

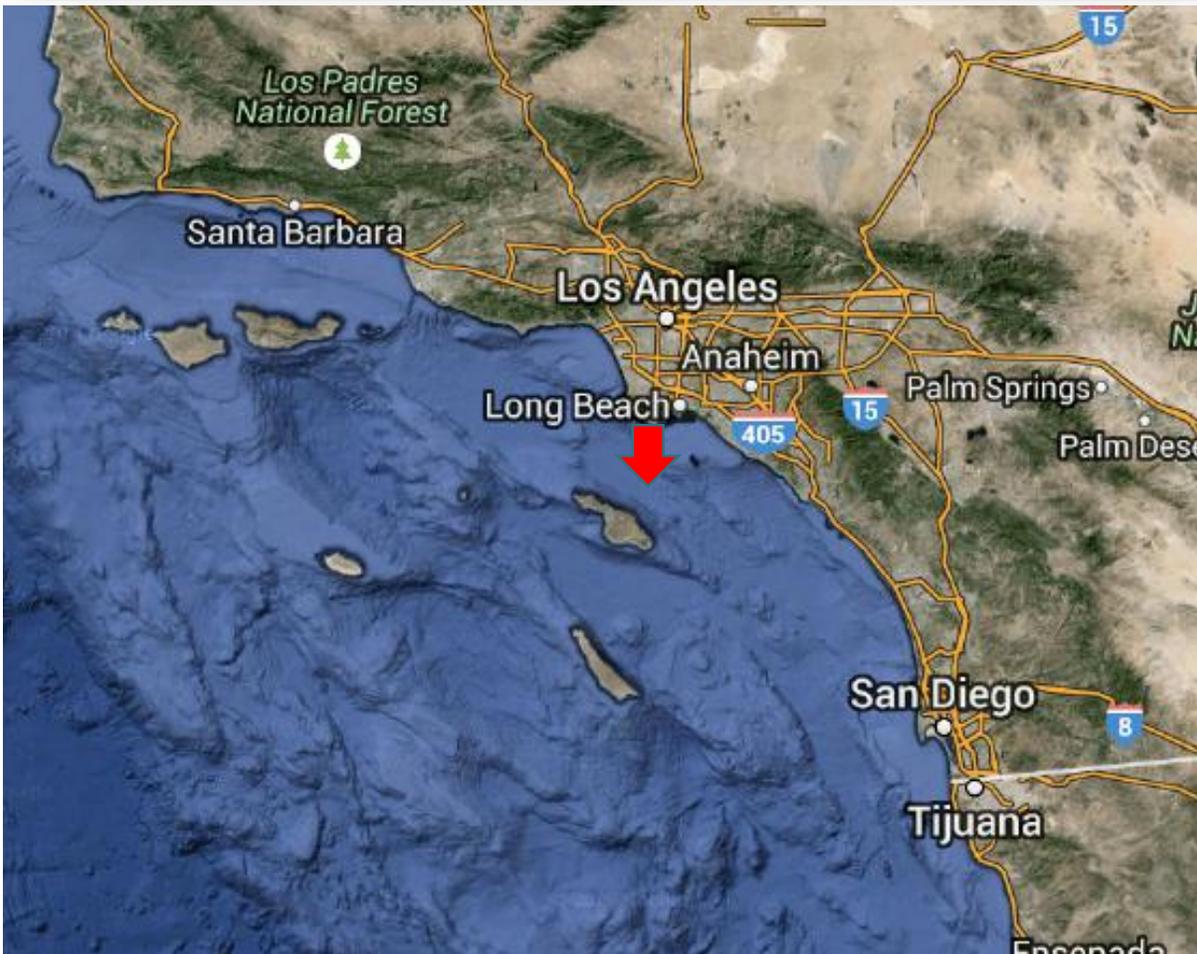
San Pedro Ocean Time-series (900m total depth)

- Monthly sampling over 5 years: Feb 2009 – Dec 2013
- Sampling at 5 depths: 5m, DCM, 150m, 500m, 890m

16S rRNA gene amplicon sequencing

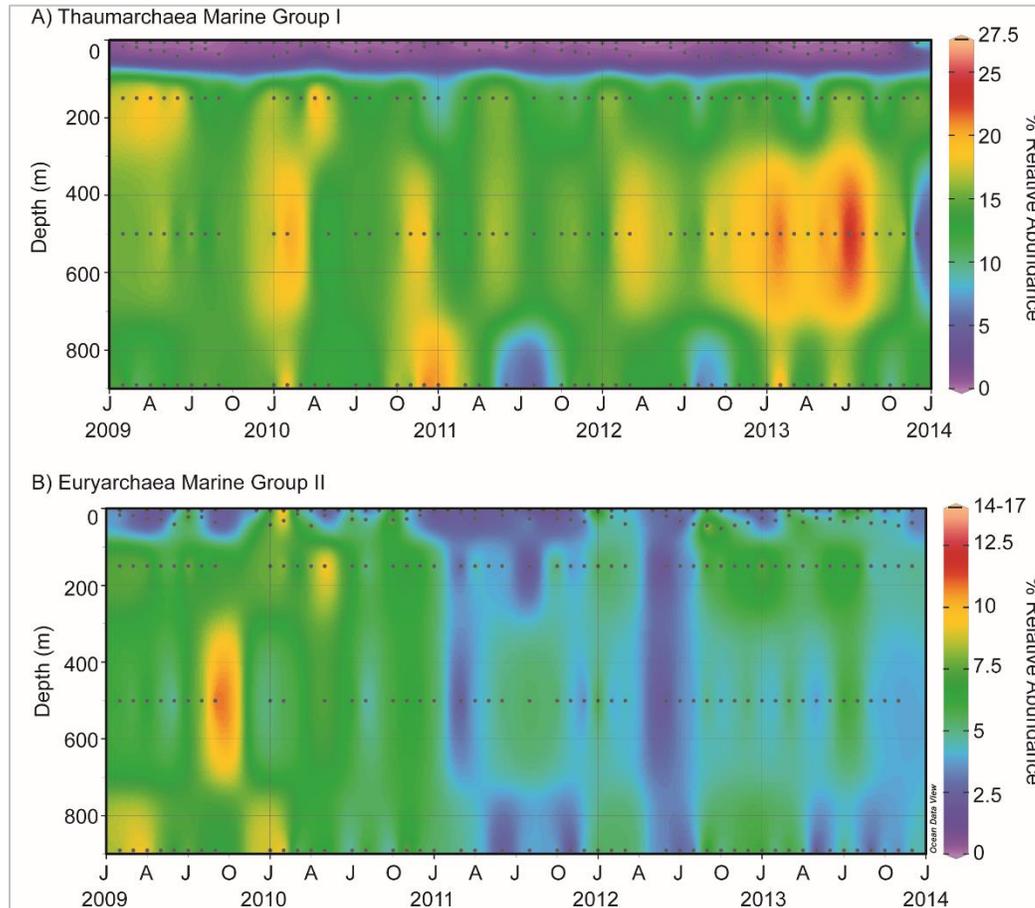
- 515F-C/926R universal primers
 - Primers target Bacteria, Archaea, Eukaryotes, chloroplasts
 - Parada AE, *et al.* (2015). *Environ Microbiol* **18**: 1403–1414.

Clustered 16S sequences by 99% similarity to evaluate the relative abundance of Thaumarchaea MGI and Euryarchaea MGII OTUs



Research Question 1:

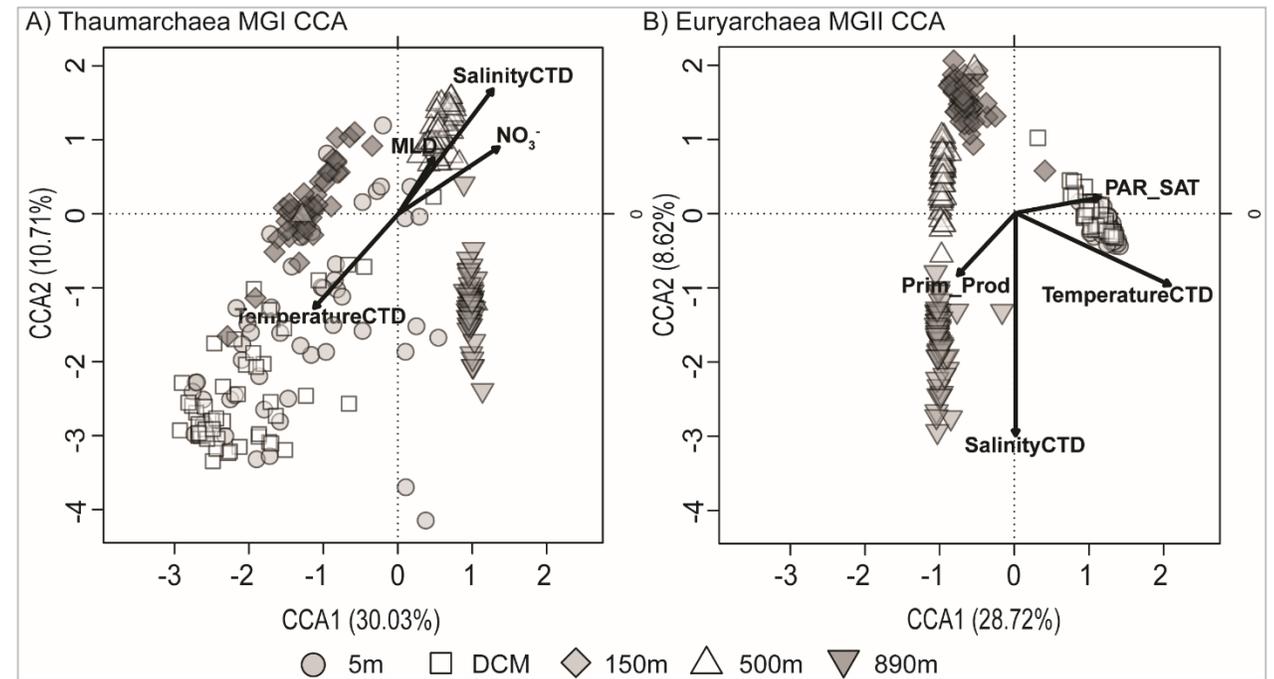
What is the distribution of the Thaumarchaea Marine Group I and Euryarchaeal Marine Group II communities across the water column?



Heatmap shows abundances over time and depth as an alternative to line graphs or barcharts.

Conclusions:

- The MGI and MGII display temporal dynamics.
- The MGI and MGII communities are stratified across the water column.



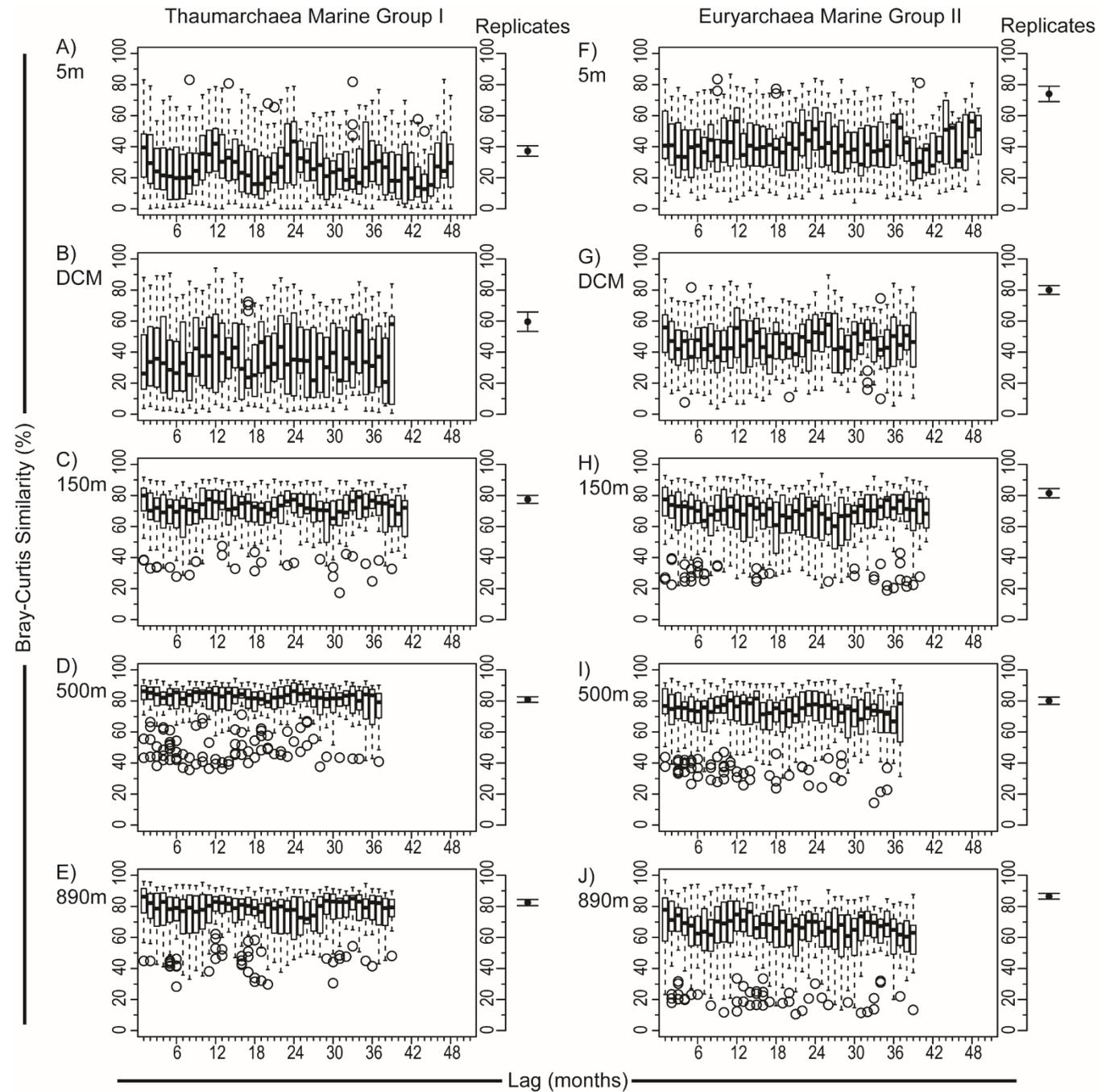
Ordination plots visually represent the similarities between communities in each sample.

Research Question 2: Does their community composition change over time?

Figure shows the community similarity between every pair of samples at different time lags

Conclusions:

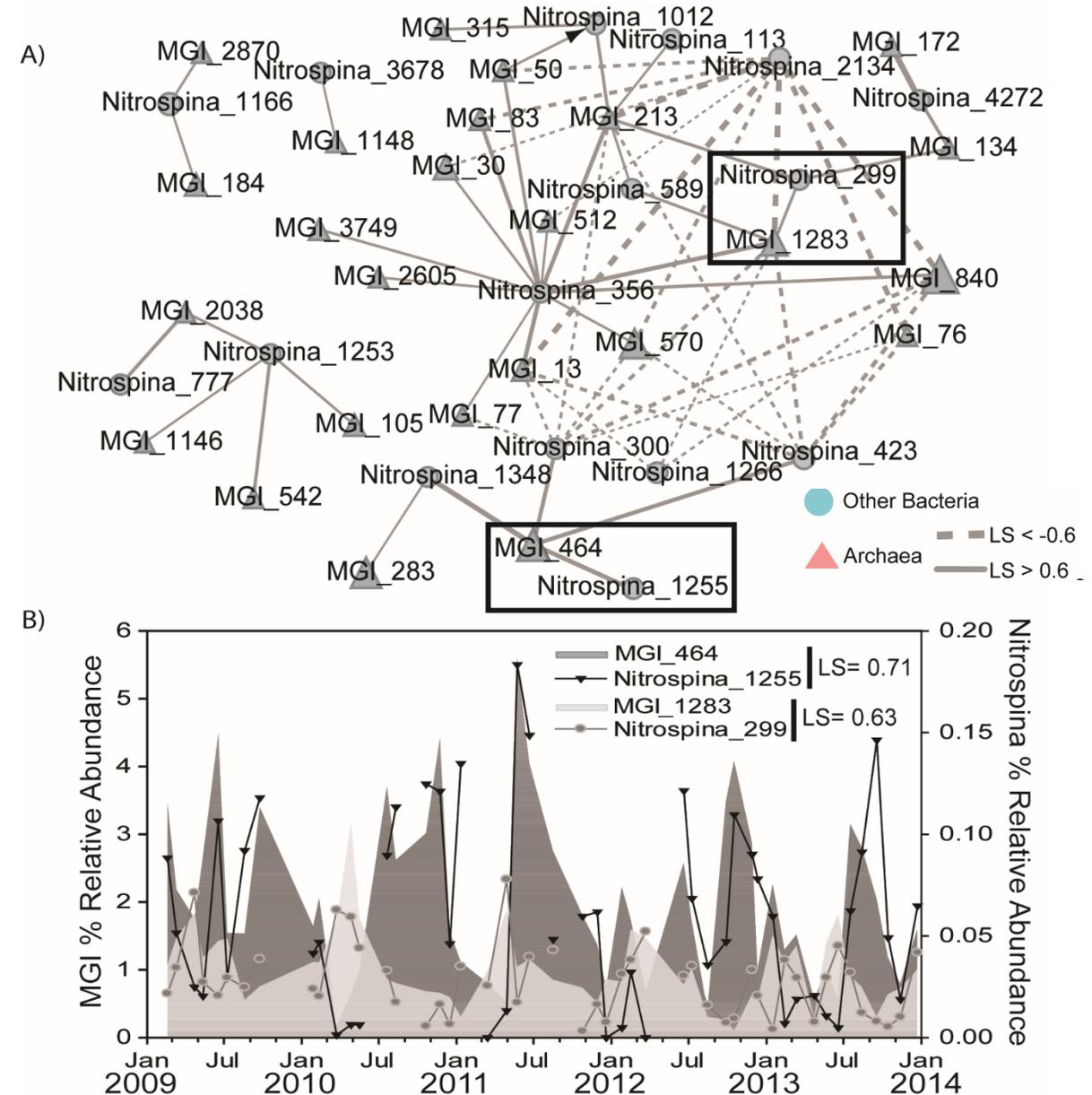
- All depths show increases in similarity at 12month intervals and decreases at 6month intervals
- Statistical tests show communities at all depths demonstrate seasonality.
- The MGI and MGII communities are seasonally variable at all depths at SPOT



Research Question 3: How do they fit into the rest of the microbial community?

Conclusions:

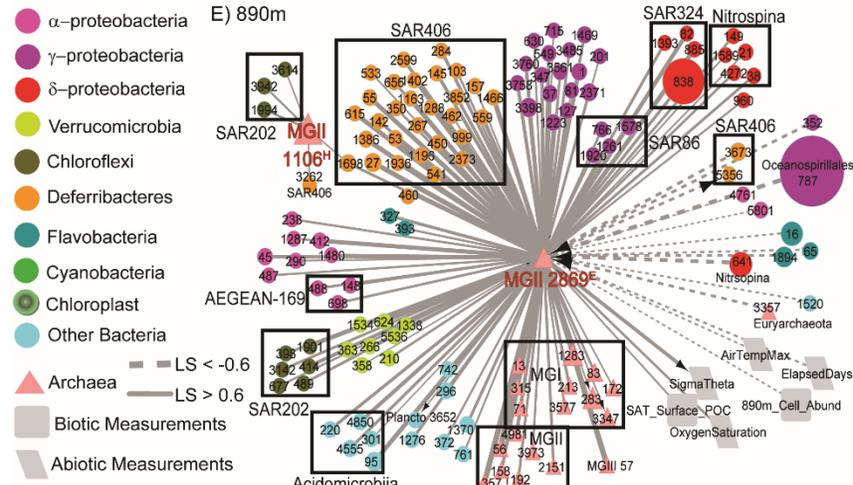
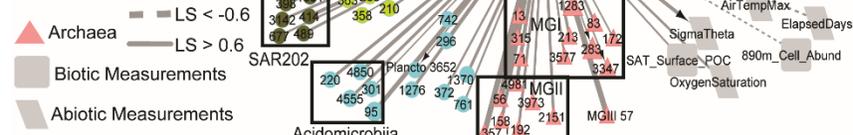
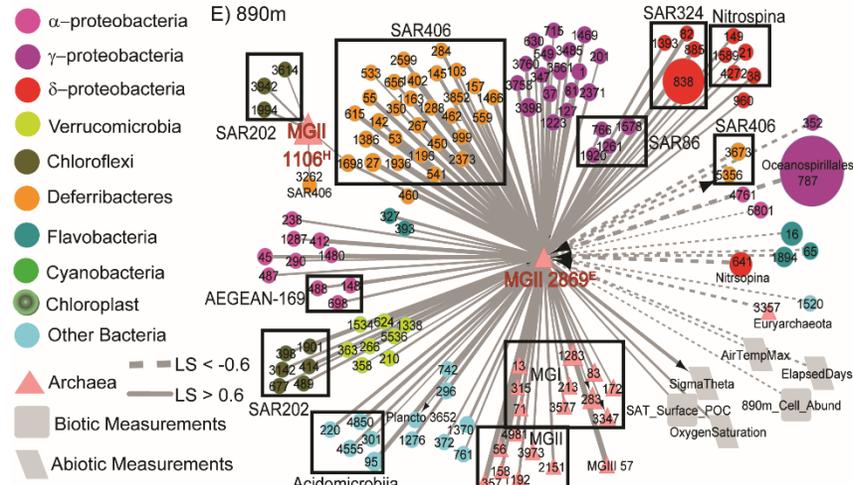
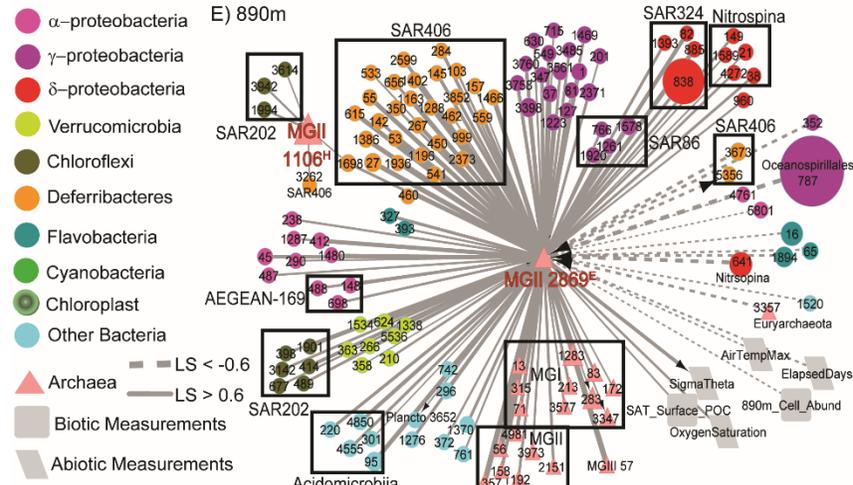
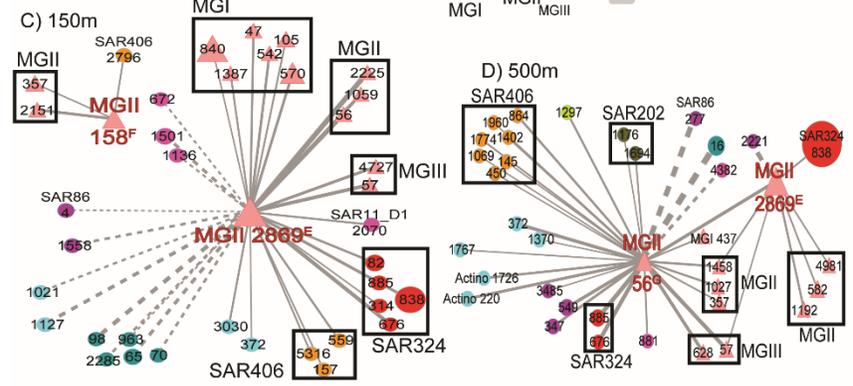
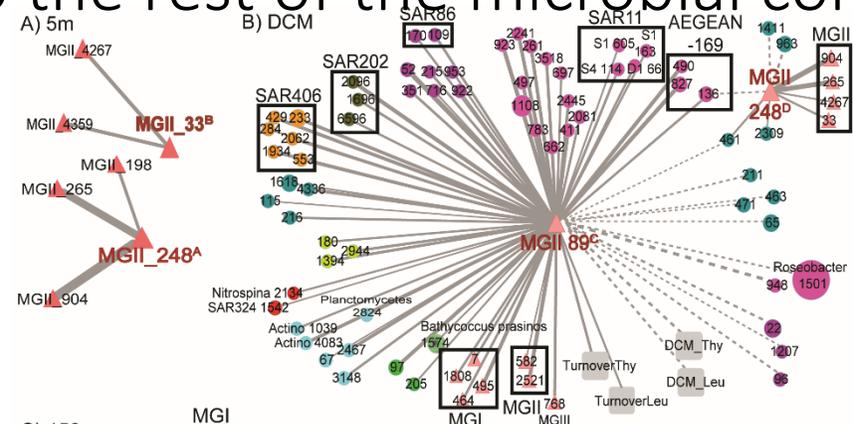
- Individual MGI are correlated to bacteria with similar function
- Despite having complimentary functions not all MGI are correlated to the same Nitrospina
- Distinct MGI/Nitrospina assemblages appear to have distinct temporal dynamics
- MGI/Nitrospina assemblages leading to global or regional changes in the nitrogen cycle



Research Question 3: How do they fit into the rest of the microbial community?

Networks of microbial OTUs and environmental variables correlated to the two most abundant MGII OTUs at each depth.

But let's focus on one depth for today!



Take home message

- The archaeal community is much more stratified at SPOT, and potentially elsewhere, than anticipated, indicating the large diversity responsible for such differences.
- Archaeal community seasonality reflects temporal dynamics of microbial assemblages, superimposed over stochastic variability.
- Changes in community structure may alter the rates or efficiencies with which the microbial community drives biogeochemical cycles.
- Understanding what selects for different assemblages may therefore be key in predicting changes to these cycles in response to regional and global environmental changes.

Dataset provided to you

- A reduced version of the OTU table I used
- Contains the % relative abundance of the most abundant OTUs
 - % is rescaled to just these OTUs
- OTU table contains information for years: 2011-2013; and depths: 5m, 150m, and 890m.

The screenshot shows an Excel spreadsheet titled "EARTH_2017_Workshop_Parada_data - Excel". The spreadsheet contains a table with the following data:

#OTU ID	Depth	OTU_depth	taxono	domair	phylum	subphy	class	order	genus	ShortName	1/11/2011	2/23/2011	3/15/2011
Otu000001	5m	Otu000001_5m	Bacteri	Bacteri	Proteol	Gamma	Oceanc	ZD0405	unclassifie	Oceanospir	0.0033548	0.0015255	0
Otu000002	5m	Otu000002_5m	Bacteri	Bacteri	Proteol	Gamma	Oceanc	unclass	unclassifie	Oceanospir	9.7524155	2.2927663	1.1906483
Otu000003	5m	Otu000003_5m	Bacteri	Bacteri	Proteol	Alphap	SAR11	Surface	unclassifie	SAR11_S1_3	6.4848363	11.955029	2.2512105
Otu000004	5m	Otu000004_5m	Bacteri	Bacteri	Proteol	Gamma	Oceanc	SAR86	unclassifie	SAR86_4	1.4023081	2.7000641	3.9043868
Otu000005	5m	Otu000005_5m	Bacteri	Bacteri	Proteol	Alphap	SAR11	Surface	unclassifie	SAR11_S1_5	0.0033548	0.0045764	0.0018068

Acknowledgments

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- Fuhrman Lab
- Myriad of people associated with running SPOT
- Funding:
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 - Gordon and Betty Moore Foundation

Questions?