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Please take 10 minutes to fill out the online course evaluation. Professor Maloof will be back shortly.

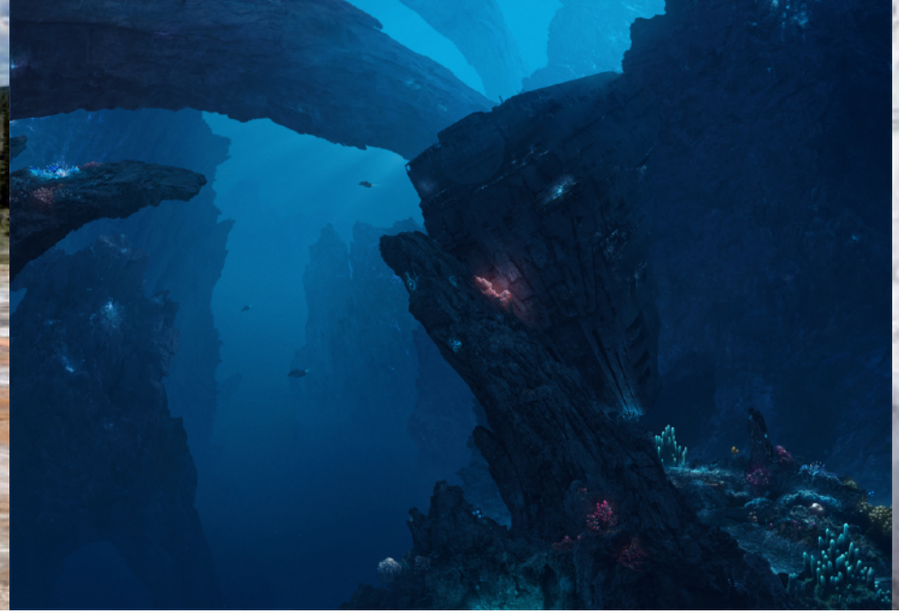
Getting Messy with Microbes:

An Introduction to Metagenomics

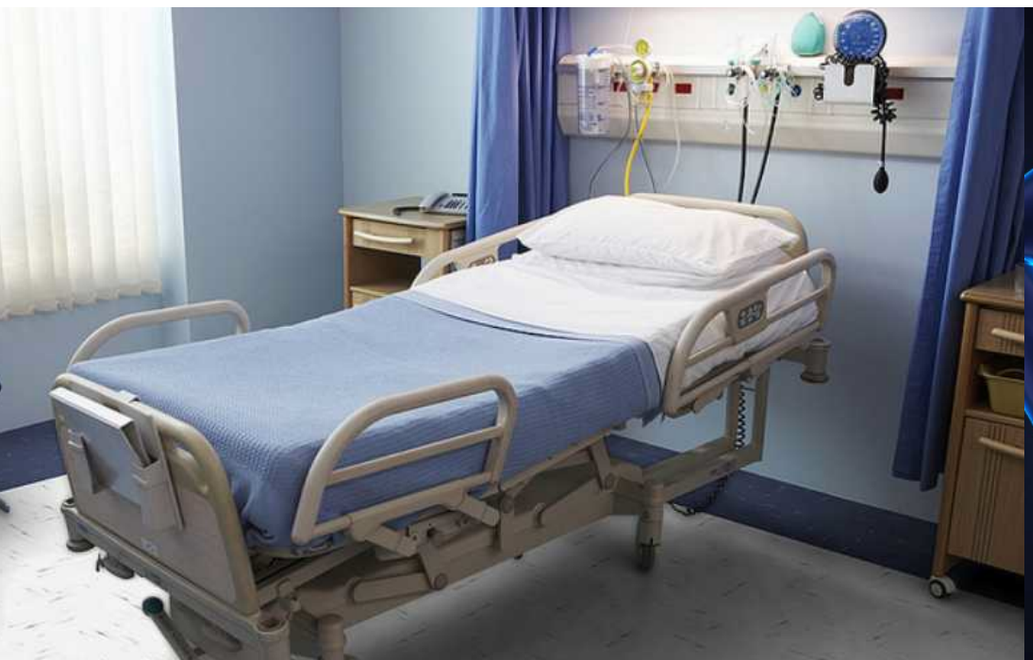
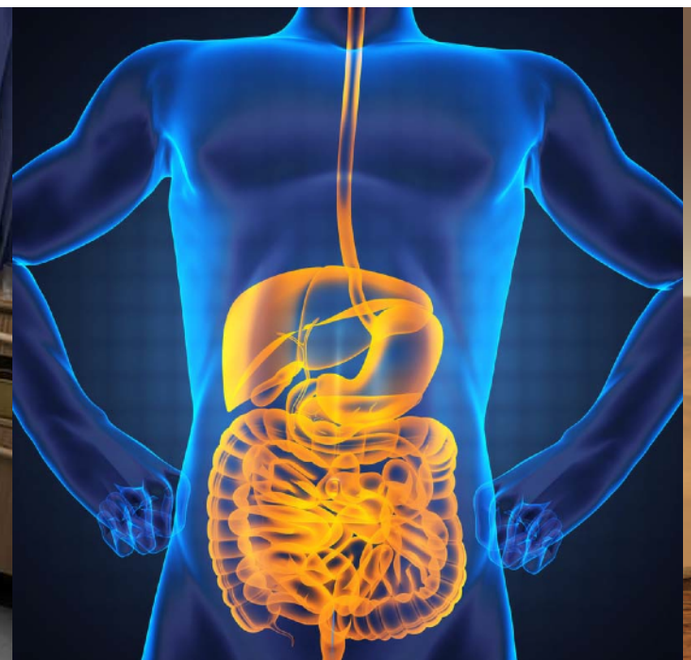
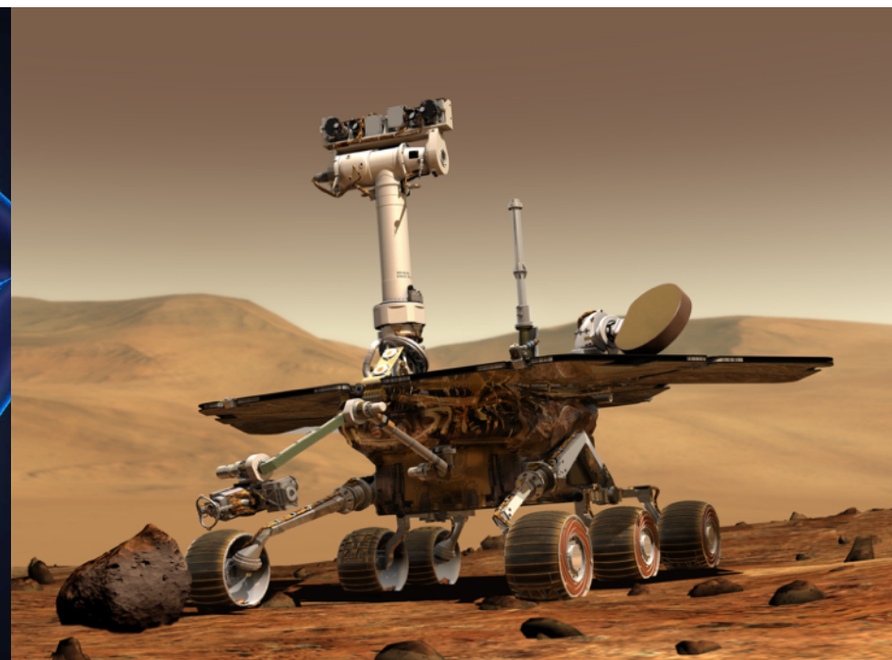
Slides from Kristen Beck, modified by Julin Maloof

Metagenomics.

The study of a collection of genetic material from a mixed community of organisms, typically microbial.


Metagenomics is a lens to observe countless environments.

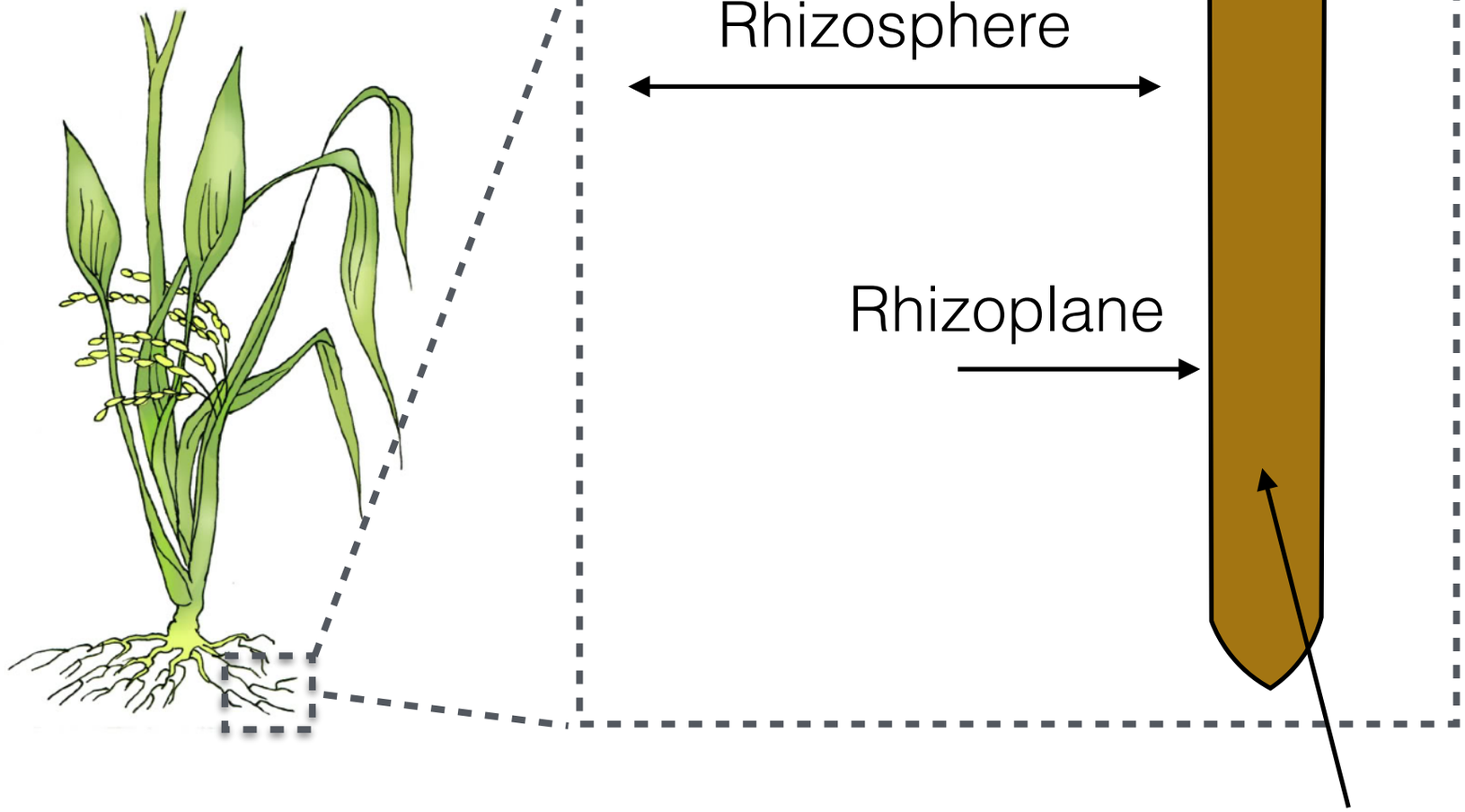
Today's lab focuses on the rice root microbiome

Photo credit: Sundaresan Lab, UC Davis

Nipponbare

M104

IR50



How do we assess microbial community composition?

Sequence the 16S ribosomal RNA

Sequencing of the 16S ribosomal RNA

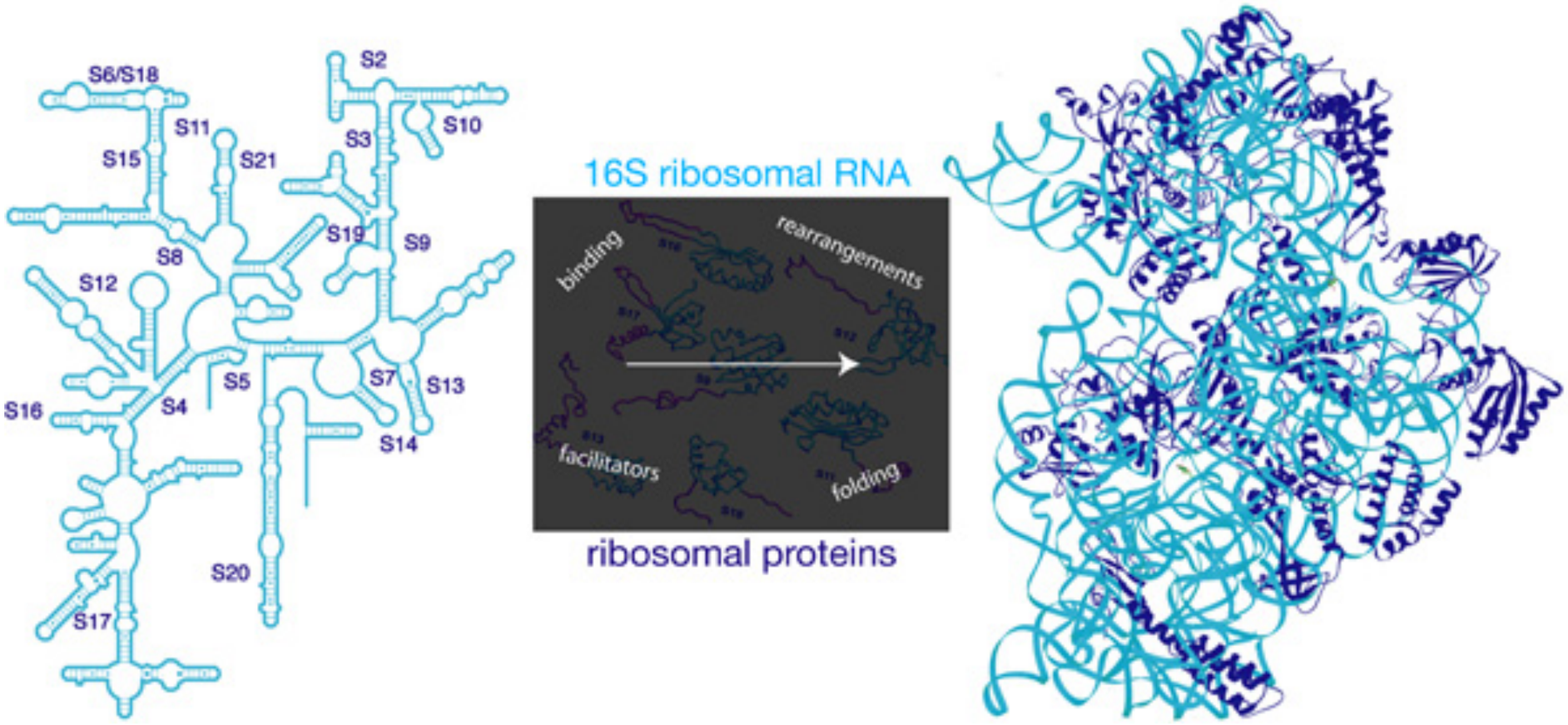


Photo credit: Culver Lab, Univ. of Rochester

What are the components of a metagenomics analysis?

Operational Taxonomic Unit (OTU)

A cluster of 16S rRNA reads with >97-99% similarity to define “species”

Questions that can be answered by 16S sequencing:

- Which microbes are in my sample? What might they be doing?
- Is microbe X present at different levels between samples (differential abundance)?
- Why might one phylum be more abundant in this environment?

Alpha Diversity

- Alpha diversity: how diverse is the microbial community?
- Can compare diversity between samples
    - e.g. is the microbial community more diverse at the root surface or in bulk soil?

Alpha Diversity Method 1

- **Count OTUs**

- Simple, but…any issues?

Alpha Diversity Method 2

- **Shannon's Diversity**

- s = number of species

$$H = -\sum_{i=1}^{s} \left(p_i \log_2 p_i \right)$$

- p_i = proportion of counts attributable to species i

- equal representation leads to high diversity index (play with it in R)

Alpha Diversity Method 3

- **Chao1**

$$chao1 = S_{obs} + \frac{F_1^2}{2F_2}$$

- *Sobs* = total observed species
- F_1 = number of singletons (species observed only once in a sample)
- F_2 = number of doubletons

Questions that can be addressed with alpha diversity:

How many taxa are in a sample? What is the richness of my sample?

Have I sequenced to a depth (coverage) that describes the diversity of my sample? (not in this lab…)

Does condition X have higher phylogenetic diversity than condition Y?

Beta Diversity

- Beta diversity measures the diversity *between* samples.

- The distance between each pair of samples (with respect to community composition) is calculated.

- There are many ways to calculate beta diversity; we will use one: Bray-Curtis

Bray-Curtis Dissimilarity

- For two samples, *i* and *j* :

$$\frac{\text{unique species counts}}{\text{total counts}}$$

- S_i and S_j = sum of counts in samples *i* and *j*

- C_{ij} = sum of min(counts) for taxa observed in both *i* and *j*

$$BC_{ij} = \frac{S_i + S_j - 2 * C_{ij}}{S_i + S_j}$$

Bray-Curtis Dissimilarity

$$BC_{ij} = \frac{S_i + S_j - 2 * C_{ij}}{S_i + S_j}$$

	Sample 1	Sample 2
Species A	5	0
Species B	4	8
Species C	7	3
Species D	10	5

- $S_i = 26; S_j = 16$
- $C_{ij} = 0 + 4 + 3 + 5 = 12$
- $BC_{ij} = \frac{26 + 16 - 2 * 12}{26 + 16} = 0.43$

Beta Diversity Questions

- Do samples contain different microbial communities?
- Which microbial taxa have increased abundance compared to another sample?
- Are there broad trends that relate many samples? Can these trends be explained by an environmental or genetic factor?