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Importance of microbiomes

Microbial Biodiversity of Soil samples

Webinar, Marts 30th, 2023

Welcome

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DANISH TECHNOLOGICAL INSTITUTE

Practicalities

- Q&A
- Chat
- Raise hand

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Master of Science
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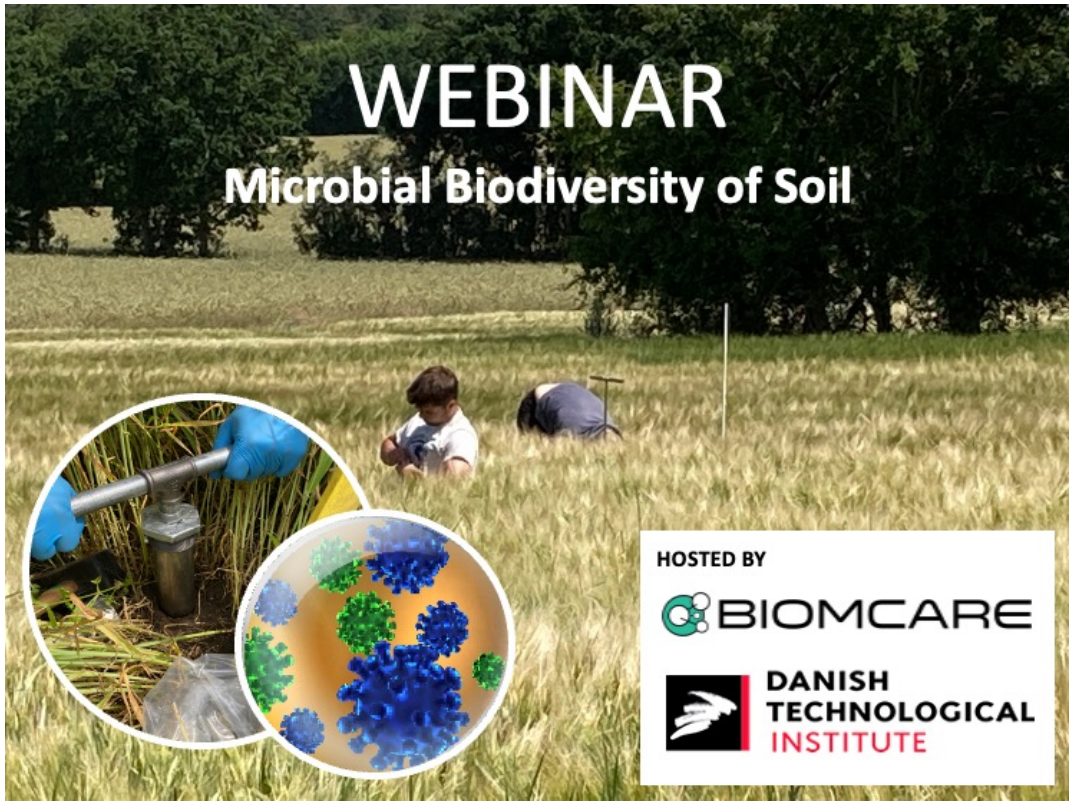
Digital & IT
MSc. in IT
Biomcare and Nioba

Agenda



Part 1 – DNA based methods for studying microbial biodiversity

- By Louise Thingholm, CEO Biomcare ApS.

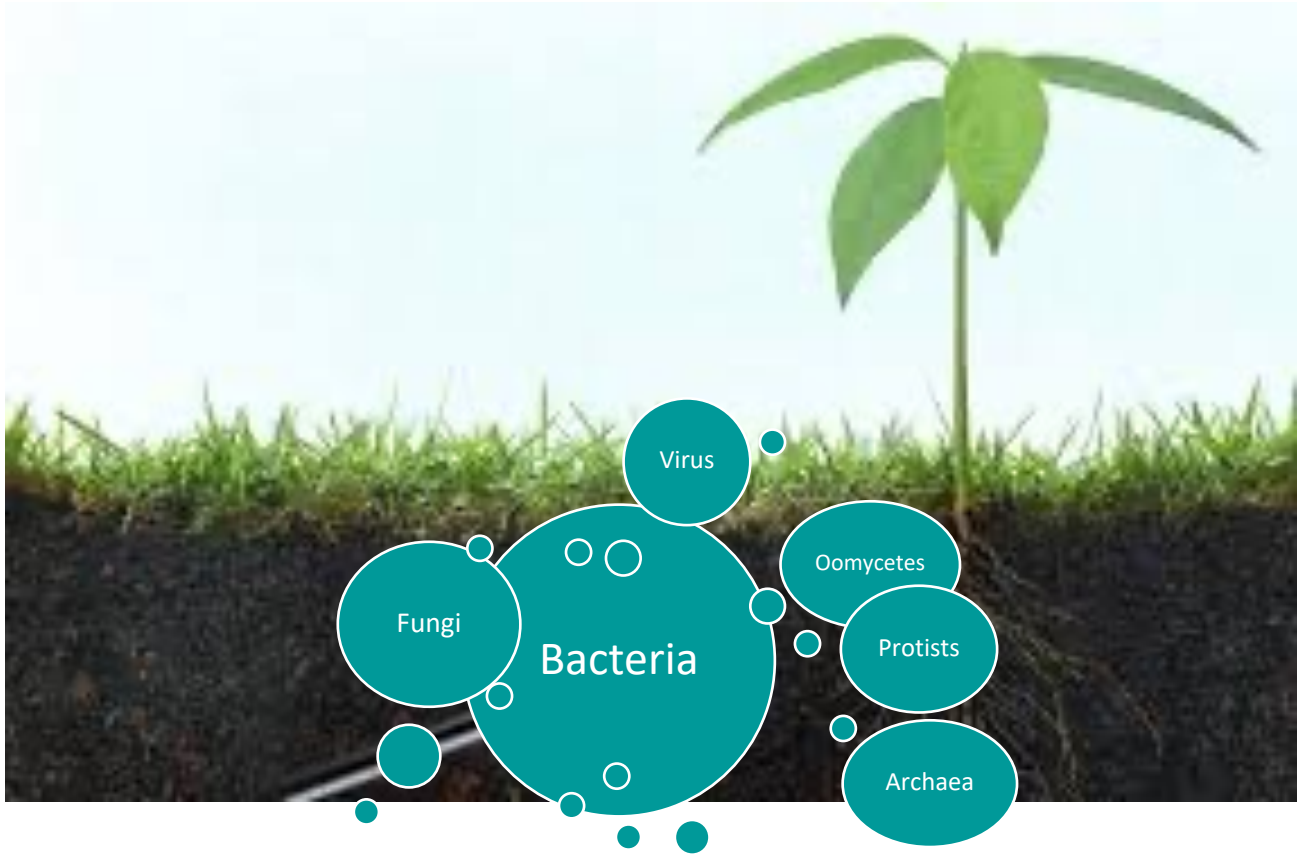
Part 2 – How soil biodiversity connects to soil health, sustainability and agricultural practices

- By Helle Hestbjerg and Valdemar Jørgensen, Danish Technological Institute.

Part 1 – Key points

1. Sequencing, what is it?
2. Why “sequencing” for studying microbial diversity?
3. Overview of sequencing types for microbiome profiling.
4. Relating biodiversity measures to other types of information.

Soil microbiome
- A complex microbial community



- >1,000 kg of microbial biomass carbon per hectare
- One of the most biodiverse habitats on Earth
- ~30,000 different taxonomic verities of microbes per spoonful of agricultural soil
- A broad diversity of microbial taxa from all three domains of life
- The majority of soil microbes remain uncharacterized

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The benefits of soil microbes

All the organisms living in soil provide benefits to the growing crop and environment. They help to:

- ✓ Decompose organic material
- ✓ Transform nutrient to a form where they are accessible to crops.
- ✓ Protect crops from pathogens and disease.
- ✓ Render the crop more resistant to stresses such as heat and drought.

What is DNA sequencing?

Based on DNA

Traditional methods

Sequencing solutions

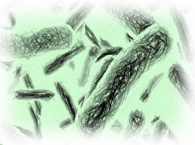
Protists

Fungi

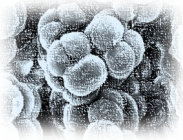
Eukaryovirus

Eukarya

Bacteria



Archeae



Archaeavirus

Archaea

Bacteria

Bacteriophage

What is DNA sequencing?

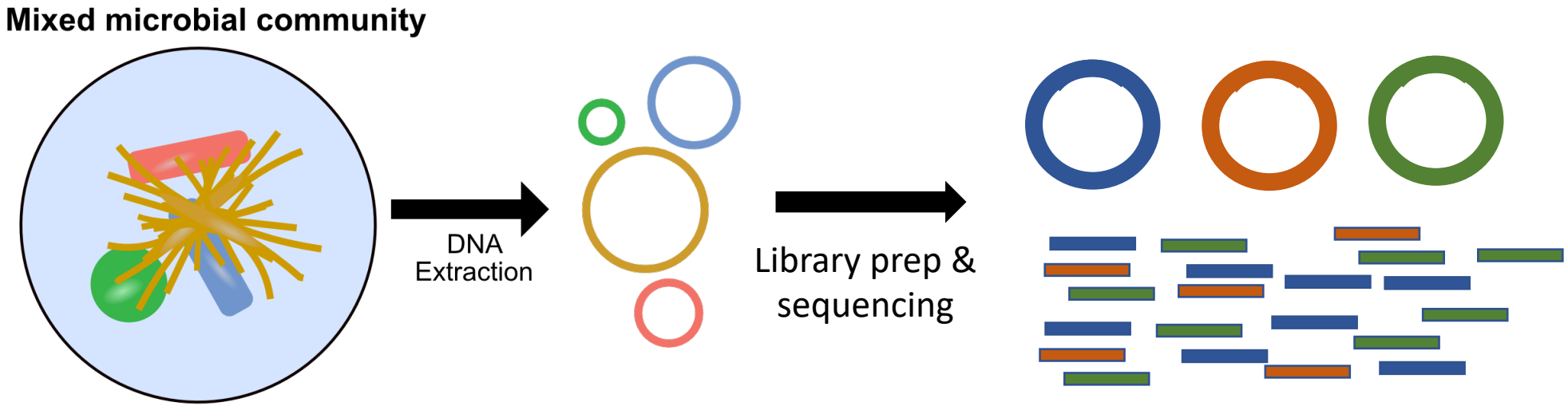


Illustration : Modified from astrobiomike.github.io

Why is sequencing a good method for studying microbial biodiversity?

Advantages

- No culturing needed / much more sensitive
- Largely unbiased / Little prior knowledge needed
- High resolution (distinguish species or even strains)
- Can detect within or across branches in the tree of life (bacteria, fungi, parasites etc.)
- Provide different types of insight into the sample microbiome composition.

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Insight into the soil microbiome biodiversity

Fastq file

Header Sequence Quality

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```

Taxonomic abundance table

Group	Taxonomy	X2014_winter_FL	X2014_winter_PA	X2(
ASV33112	Bacteria(100);Proteobacteria(100);Alphaproteobacteri...	1793	152	
ASV122970	Bacteria(100);Cyanobacteria(100);Cyanobacteria(100);...	492	112	
ASV148428	Bacteria(100);Proteobacteria(100);Gammaproteobacte...	0	252	
ASV212114	Bacteria(100);Cyanobacteria(100);Cyanobacteria(100);...	574	184	
ASV9620	Bacteria(100);Proteobacteria(100);Alphaproteobacteri...	464	70	
ASV147186	Bacteria(100);Proteobacteria(100);Betaproteobacteria(...	0	40	
ASV89359	Bacteria(100);Proteobacteria(100);Alphaproteobacteri...	142	8	
ASV1061	Bacteria(100);Proteobacteria(100);Gammaproteobacte...	0	4	
ASV328581	Bacteria(100);Bacteroidetes(100);Bacteroidia(100);Bac...	2	540	
ASV86104	Bacteria(100);Proteobacteria(100);Alphaproteobacteri...	72	6	
ASV57649	Bacteria(100);Proteobacteria(100);Alphaproteobacteri...	69	24	
ASV172568	Bacteria(100);Proteobacteria(100);Gammaproteobacte...	130	7	
ASV237646	Bacteria(100);Bacteroidetes(100);Flavobacteriia(100);F...	0	0	
ASV67428	Bacteria(100);Planctomycetes(100);OM190(100);OM1...	0	16	

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Insight into the soil microbiome biodiversity

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GAGGATCCCAGGGAGGAAGGTCCTCGCTCCTCTTTCATCTAAGGGA
+
12BAFB?A:3<AE1@<FF;1*@EG*)?0?DBD>9BF9B*?######
@HWI-ST227:389:C4WA2ACXX:8:2208:2467:44624
AAAGAGGAGAGAGGACCATCCTCCCTGGGATCCTCAGAAGTCTACT
+
BDDA:DB?2AA@FC>F?EEGC<FED>GFD;?GBB?<?F99*/9?9?
```
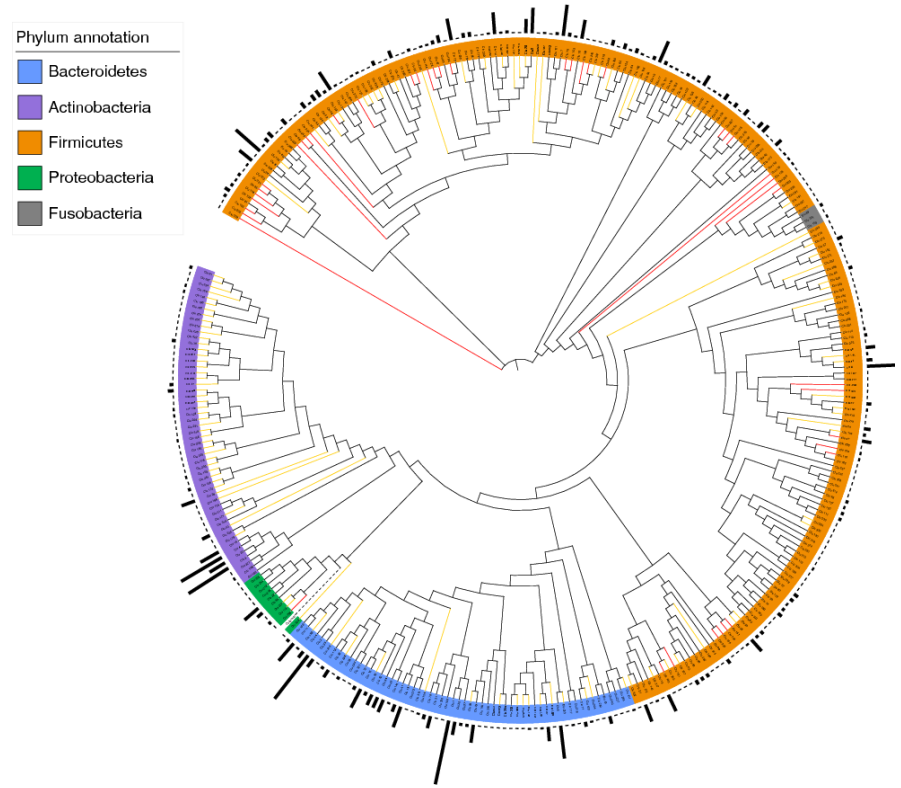

Taxonomic profile



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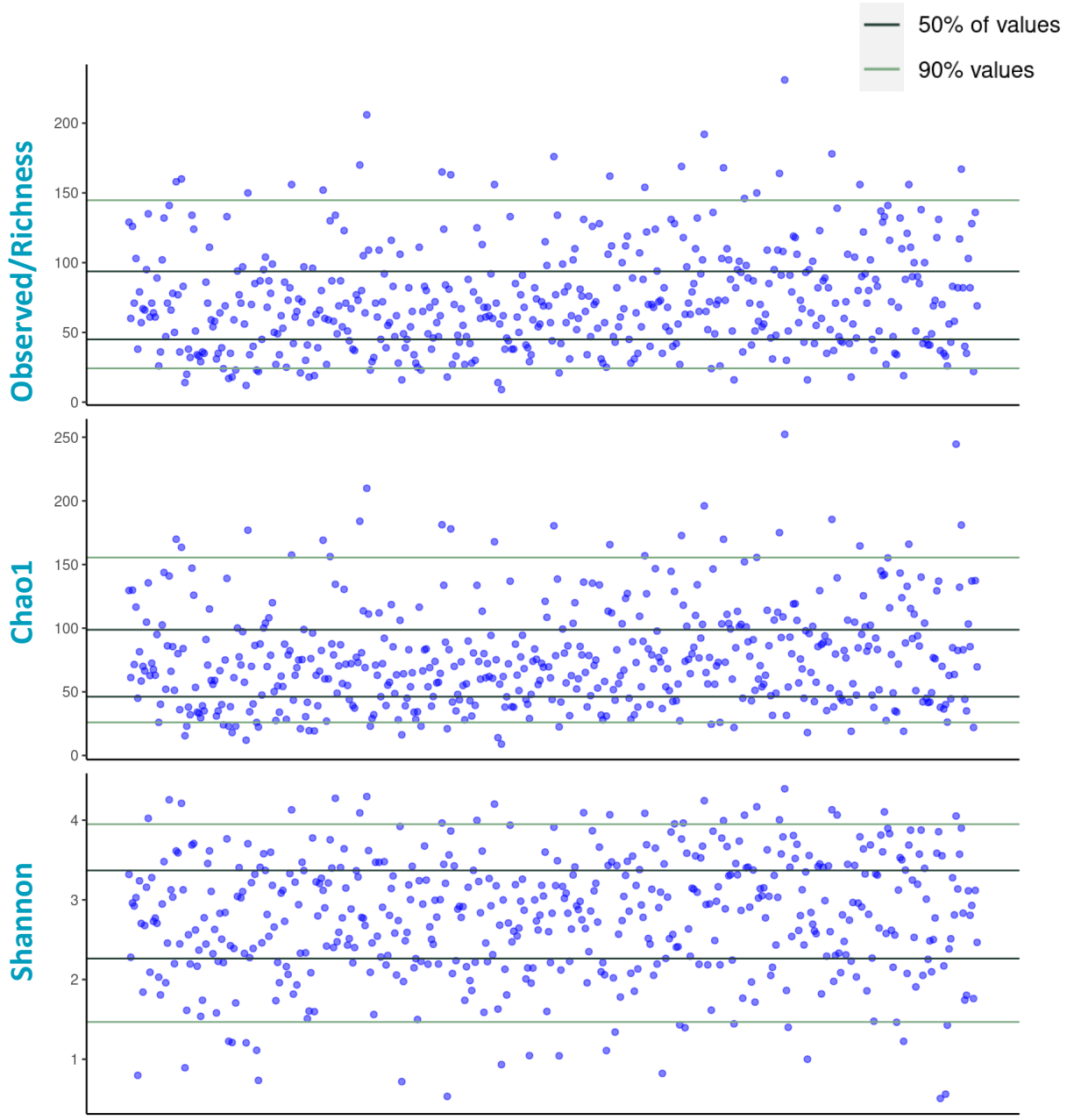
Biodiversity

“Alpha-diversity”

- A measure of the biodiversity of a habitat, considering richness, evenness or a combination of those properties.
- Calculated based on the taxonomic abundance table
- Common examples are: Richness/observed, Shannon, Faith’s phylogenetic diversity, Chao1, Simpson index.
- Each of these alpha diversity measures provides a different perspective on the diversity of microbial species within a sample



Biodiversity

“Alpha-diversity”

- **Richness**: Richness is a measure of the number of different microbial species present in a sample. It is a simple and straightforward metric that is easy to interpret, but does not take into account the relative abundance of different species.
- **Shannon index**: The Shannon index takes into account both the number of species present in a sample, as well as their relative abundances. It is a more complex metric than richness, but provides a more complete picture of the diversity within a sample.
- **Simpson index**: The Simpson index is another metric that takes into account both the number of species and their relative abundances. It is similar to the Shannon index, but tends to give more weight to the most dominant species in a sample.
- **Faith's phylogenetic diversity**: Faith's phylogenetic diversity is a metric that takes into account the evolutionary relationships between different microbial species within a sample. It is based on the idea that species that are more distantly related contribute more to overall diversity than closely related species.
- **Chao1**: Chao1 is a commonly used alpha diversity metric in microbiome research that estimates the richness, or number of unique microbial species, within a sample. Unlike other richness estimators, Chao1 takes into account the number of rare species that are present in only one or a few samples, and estimates the total number of unique species present in the sample, including those that may not have been observed.

Different Sequencing Methods for microbiome profiling

Illustration : Modified from astrobiomike.github.io

Sequencing methods for microbiome profiling

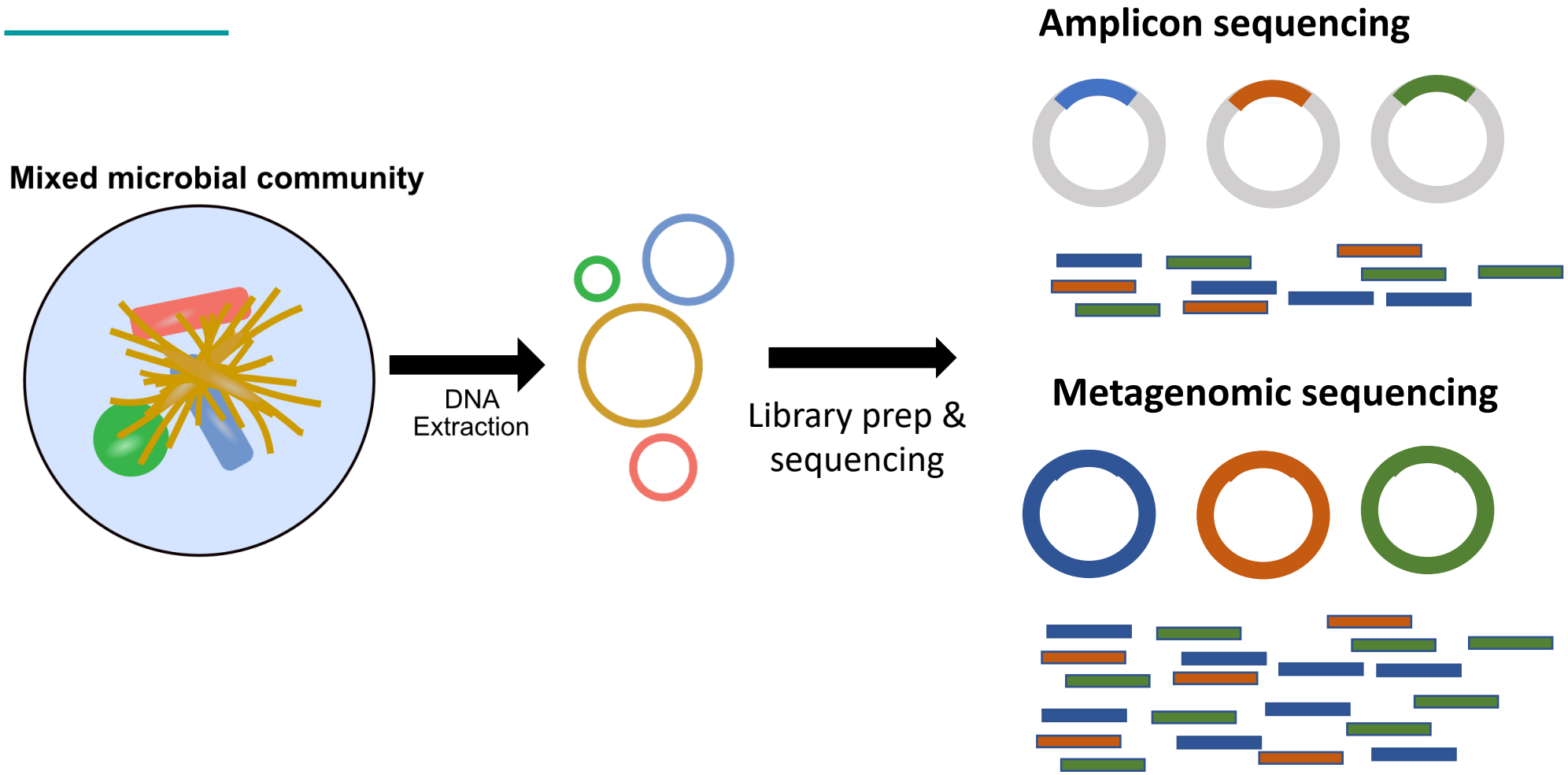
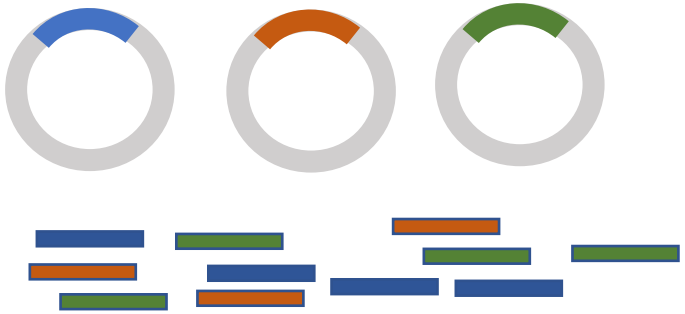


Illustration : Modified from astrobiomike.github.io

Sequencing methods for microbiome profiling

Amplicon sequencing



Marker genes

Bacteria – 16S rRNA

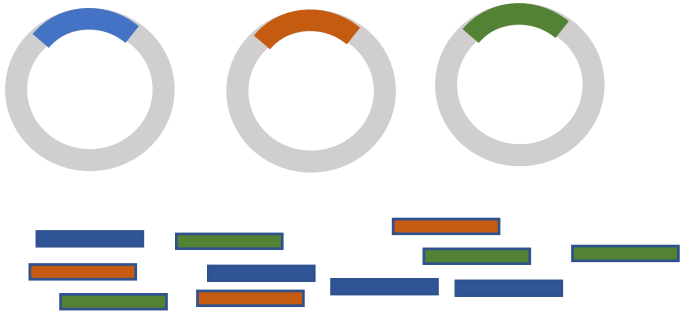
Fungi – ITS or 18S rRNA

Archaea – 16S rRNA

Metagenomic sequencing

(Shotgun)

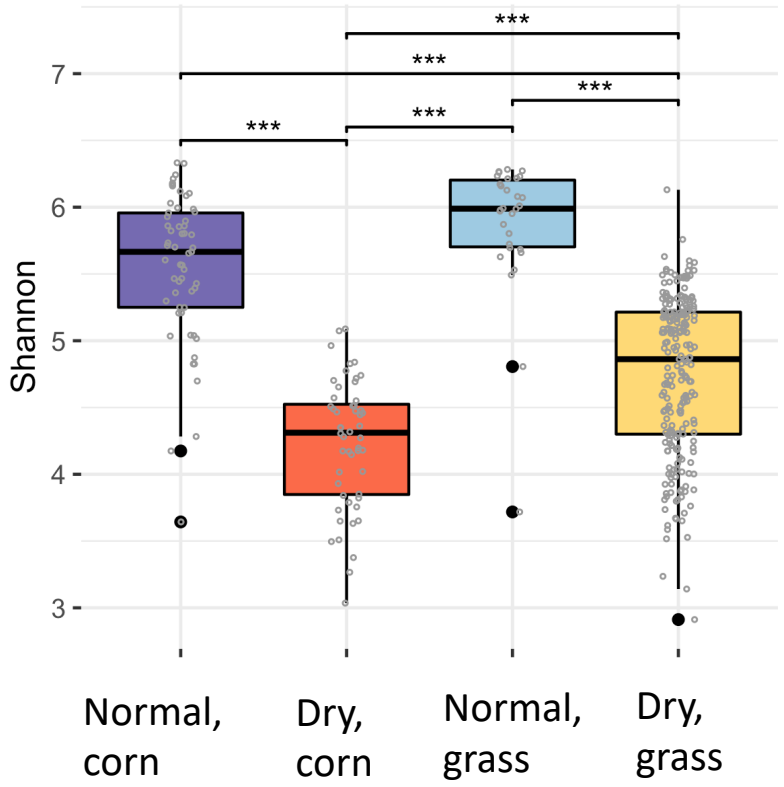
Sequencing methods for microbiome profiling

	Types of insight gained	Biodiversity
Amplicon sequencing 	Taxonomic profiling • Phyla to species • Unannotated clades	• Highly sensitive • Subset of microbiome (bacteria, fungi, parasites etc.) • Include unknown organisms • Lower resolution (often genus level) • Cheaper
Metagenomic sequencing 	Taxonomic profiling • Domaine to sub-species • (Unknown organism genomes) Functional capacity • Gene abundance • Pathway abundance • Taxa-assigned function	• Less sensitive • All microbiome (bacteria, fungi, parasites etc.) but more seq. depth sensitive. • Include known organisms (for complex communities) • Higher resolution resolution (species or even strain) • More expensive

Would you like us to host a 3–4-hour workshop on the subject?

- Yes
- No
- Maybe / don’t know

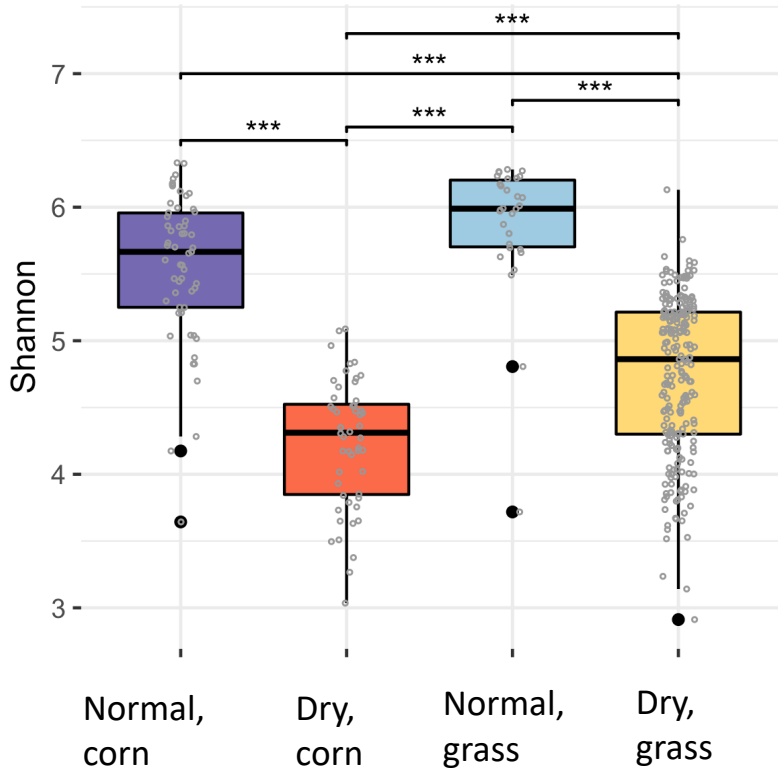
Interpreting Biodiversity Results



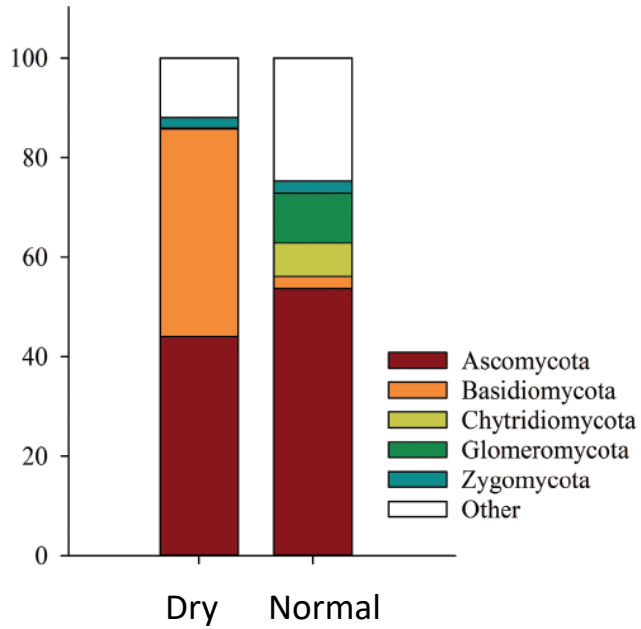
Meta-data

- Study design
- Field
- Soil type
- pH
- Crop
- Yield
- Fertilizer
- …

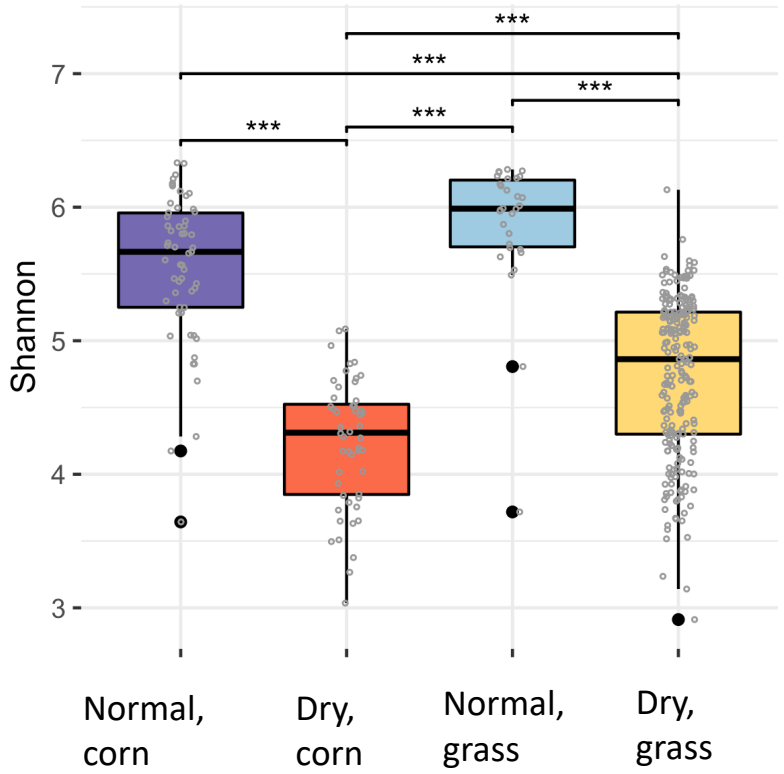
Interpreting Biodiversity Results

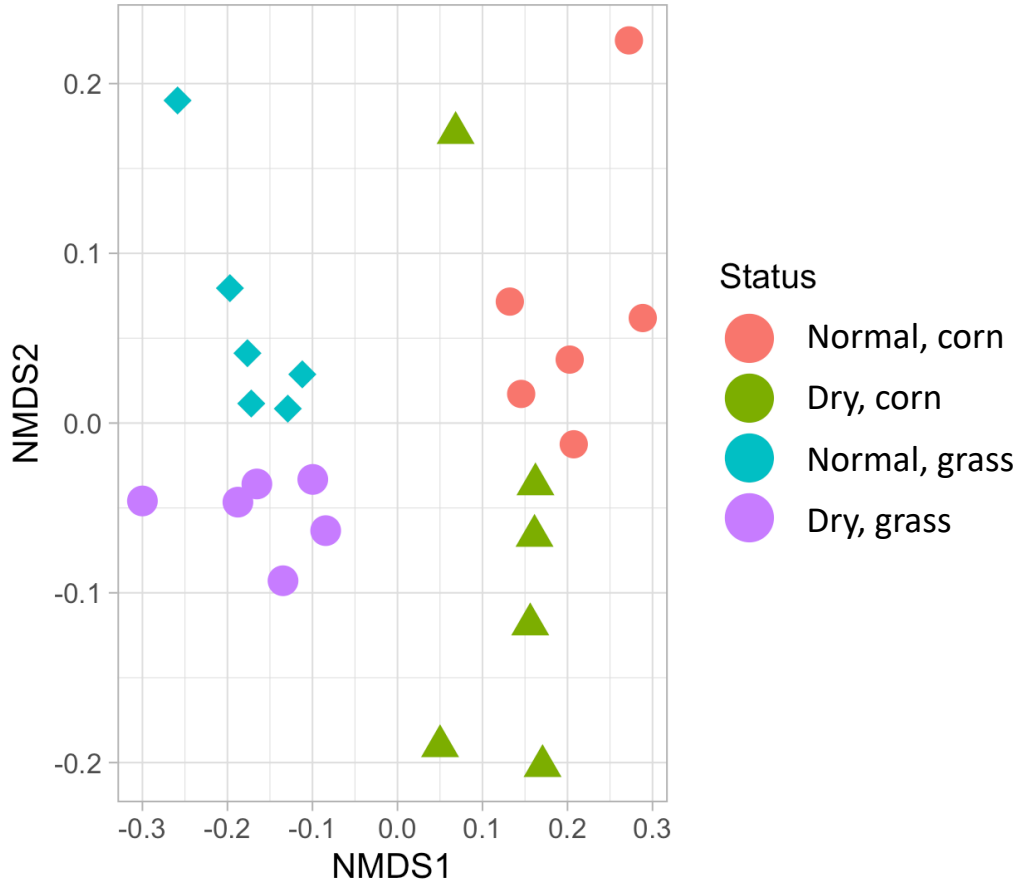


Changes in single organisms



Interpreting Biodiversity Results





Modified figure, with usage permission from SEGES

Interpreting Biodiversity Results

Functional capacity

- Gene abundance
- Functional categories (KEGG, EC, KO)
- Pathway abundance
- Taxa-assigned function

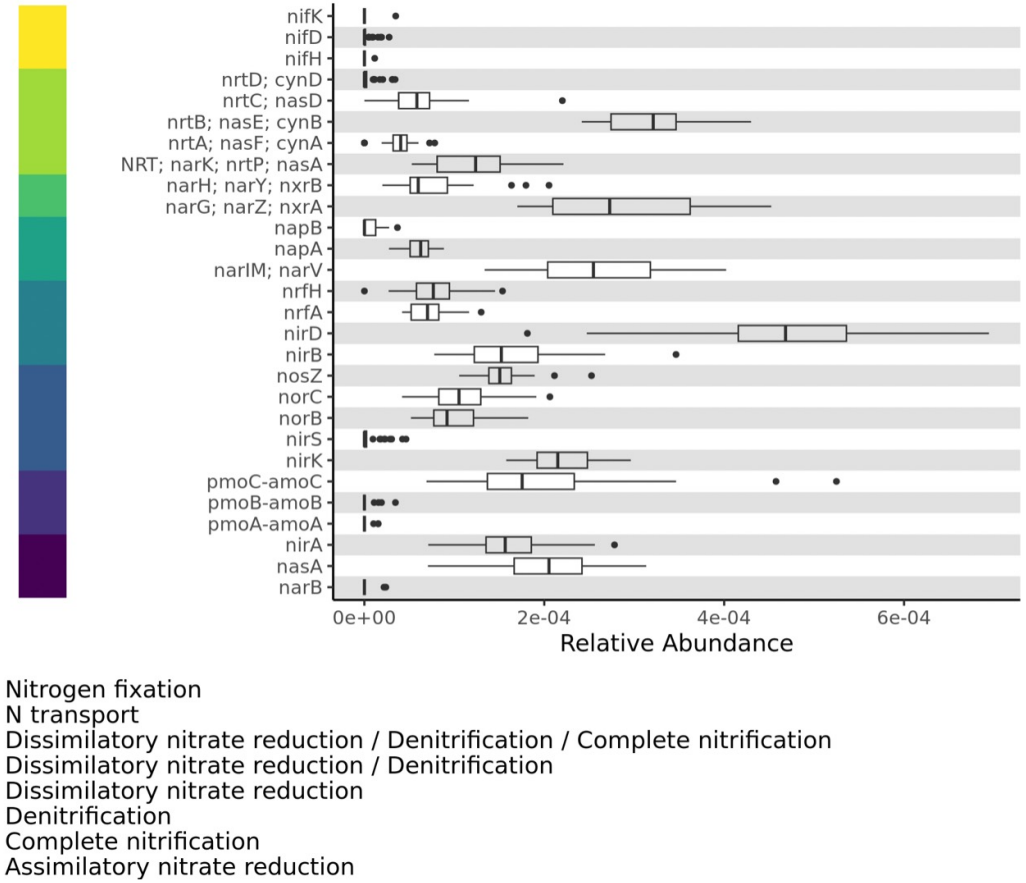
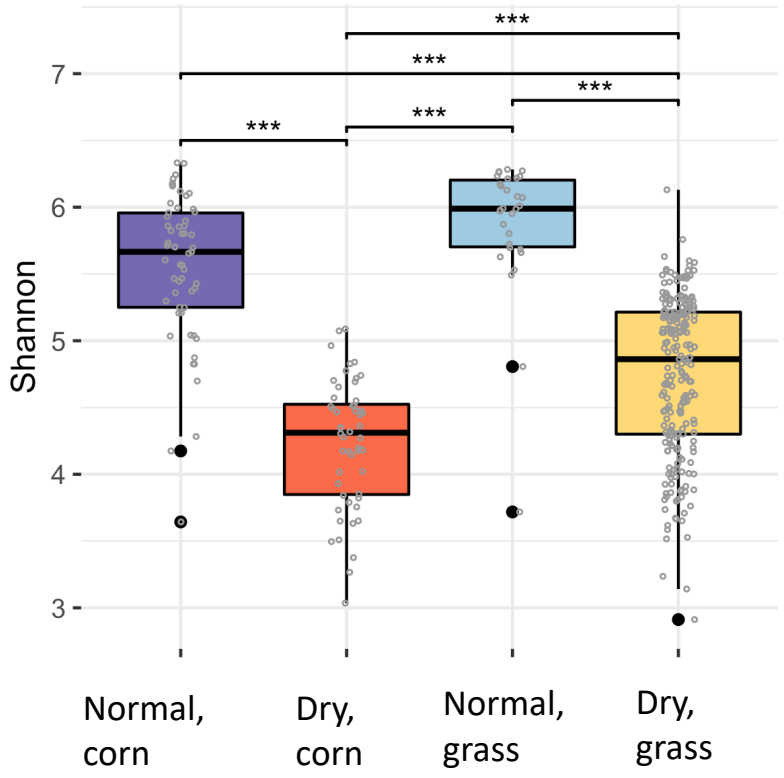


Figure 1: Boxplot of selected KO terms found in Nitrogen metabolism. The colored bar indicates which KEEG module each KO belongs to. Each row in the boxplot is one KO group (some groups have multiple names as seen in the figure). Furthermore, some KOs are found in several modules as indicated in the legend of the colored bar.

Interpreting Biodiversity Results



- ✓ Meta-data
- ✓ Changes in Single taxa
- ✓ Shifts or community level changes
- ✓ Functional capacity
- ✓ F/B ratio

Modified figure, with usage permission from SEGES

Getting started with soil microbiome analysis

Collecting samples

- Download our free sample-collection guide (https://biomcare.com/info/sample-guides-free/)
- Be consistent (method, depth, storage etc.)
- Stabilize or freeze: Plan for the aim (DNA or RNA), logistics and feasibility
- Limit contamination

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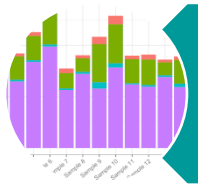
Biomcare – Your Microbiome Department on Demand

Bringing the value of sequencing-based solutions to everyone

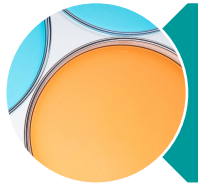
Big and small
With and without prior experience

Our services at a glance

CONSULTING AND WORKSHOPS



MICROBIOME PROJECTS A-Z



CULTURE GENOMICS

PATHOGEN DETECTION

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Confounders

- Collect as much information as possible
- Same variables not important across settings
- Different taxa respond to different factors
- Factors are interdependent and interact with the microbiome

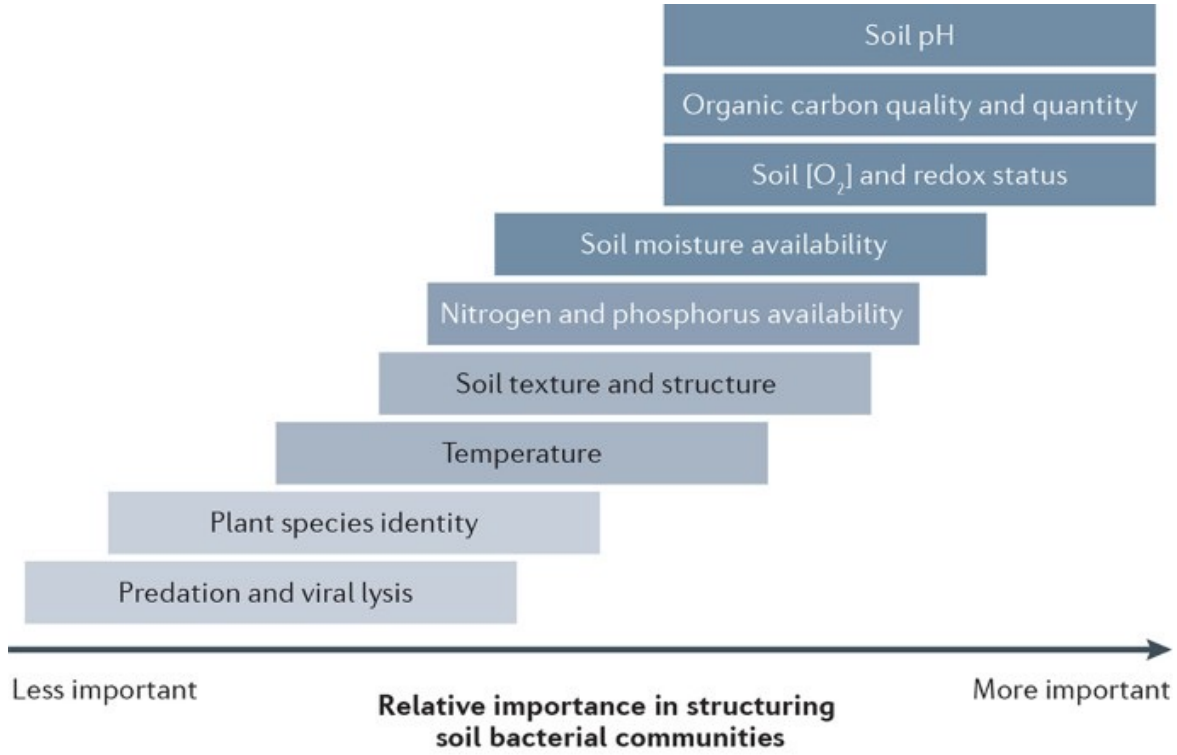

Image from Noah Fierer review, 2012

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