

# MICROBIOMA E MICROBIOTA

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# Concept maps vs mental maps

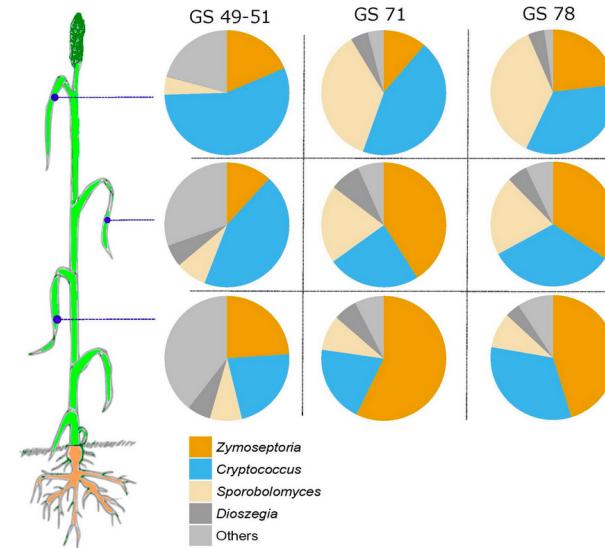
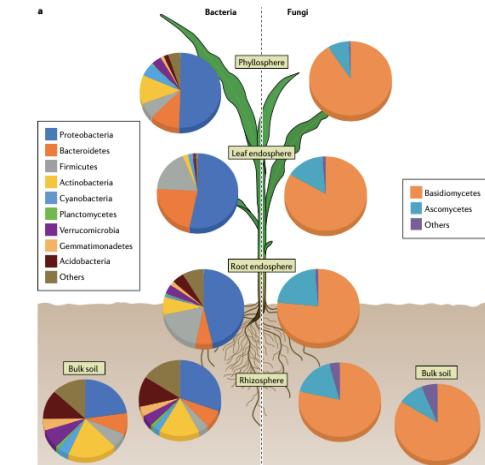
# Last lesson recap

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1. Plant microbiota changes spatially and temporally
2. Plant compartment is a major selective force that shapes the composition of plant-associated microbiota
3. Plants and their associated microorganisms form a holobiont
4. Core and Hub microbiota

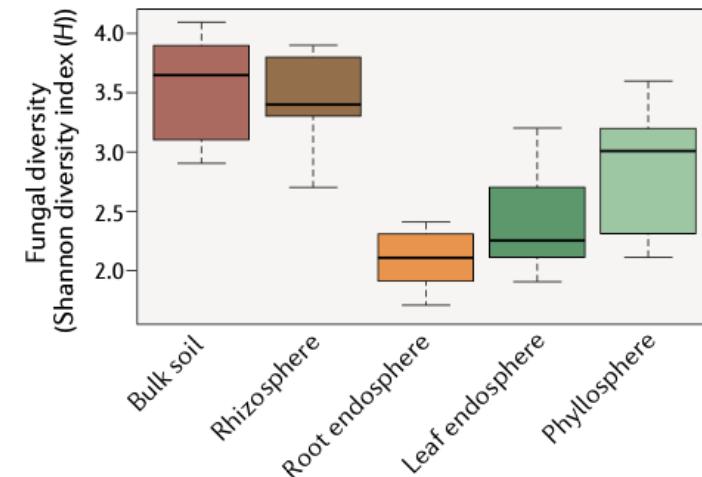
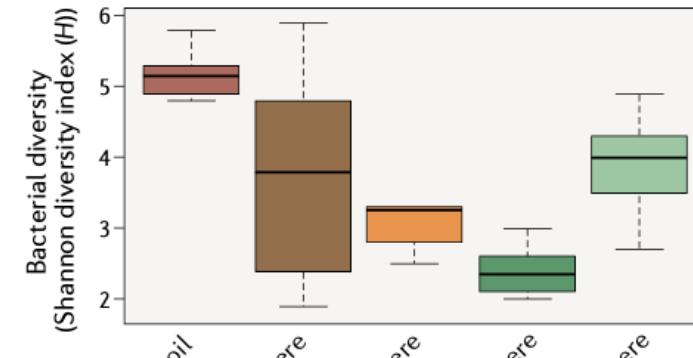
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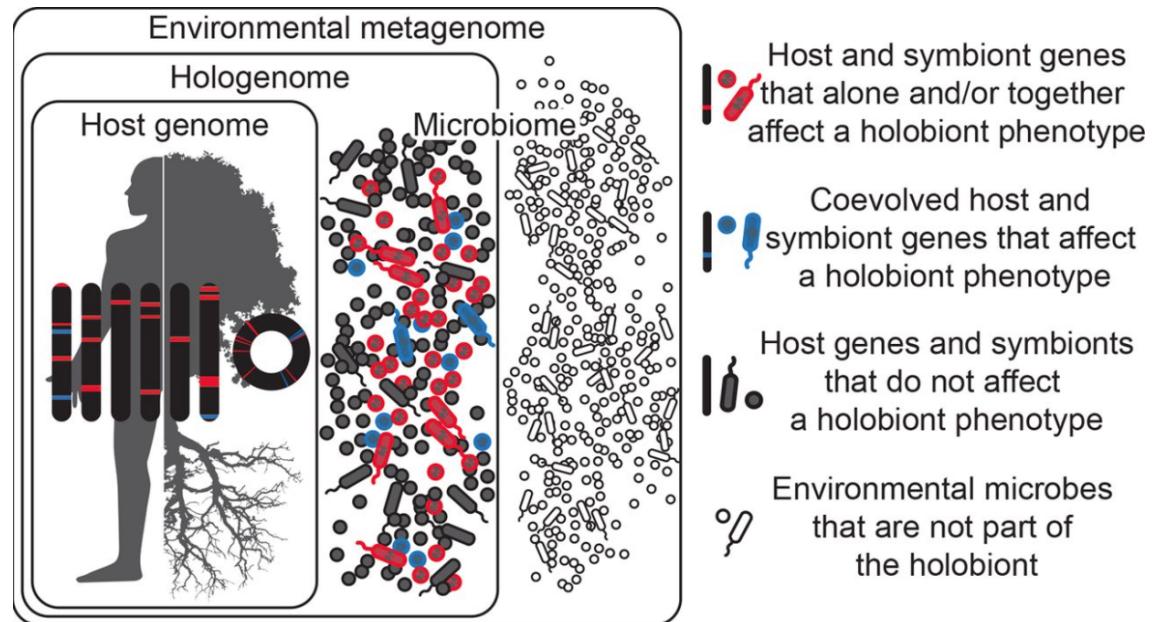
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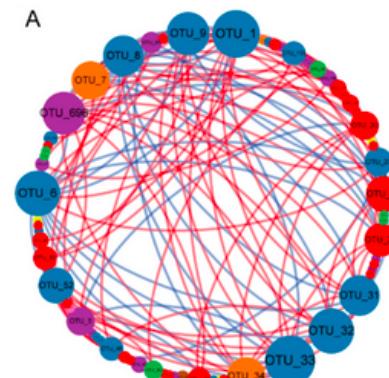
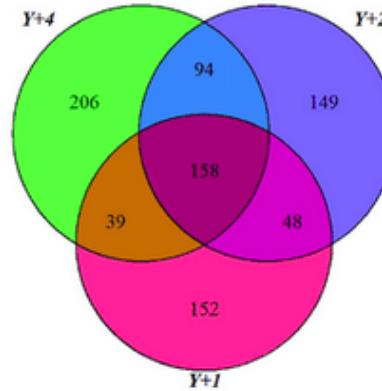
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# Core microbiota

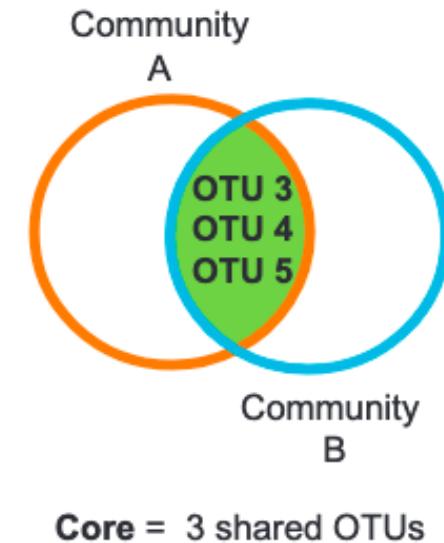
# Core microbiota

## A. Membership:

shared OTU occurrences across communities

1 = present, 0 = below detection

List of observed OTUs	Occurrences in community A	Occurrences in community B	Shared occurrences A & B
OTU 1	1	0	
OTU 2	0	1	
OTU 3	1	1	X
OTU 4	1	1	X
OTU 5	1	1	X



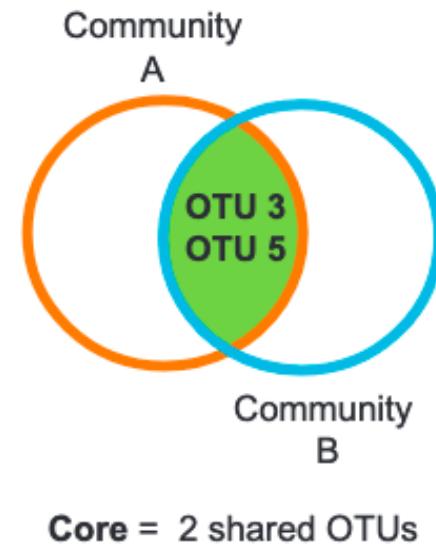
(Shade et al., 2011)

# Core microbiota

## B. Composition:

similar OTU abundances across communities

List of observed OTUs	Abundances community <b>A</b>	Abundances community <b>B</b>	Similar abundances <b>A &amp; B</b>
	OTU 1	0.4	0
OTU 2	0	0.1	
OTU 3	0.1	0.1	X ●
OTU 4	0.2	0.5	
OTU 5	0.3	0.3	X ●

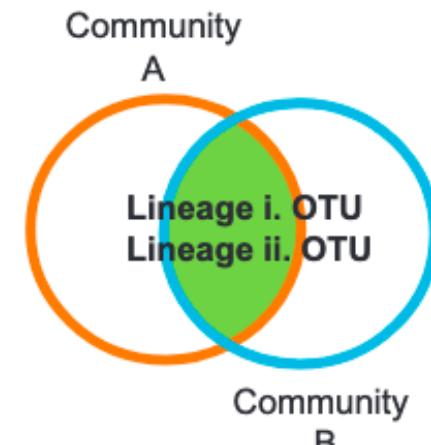


(Shade et al., 2011)

# Core microbiota

C. Phylogeny:  
shared OTU lineages across communities

lineage	Abundances		Similar abundances <b>A &amp; B</b>
	community <b>A</b>	community <b>B</b>	
i.	OTU 1 0.4	0	☒ X ●
ii.	OTU 2 0	0.1	☒ X ●
iii.	OTU 3 0.1	0.1	
	OTU 4 0	0.8	
	OTU 5 0.5	0	



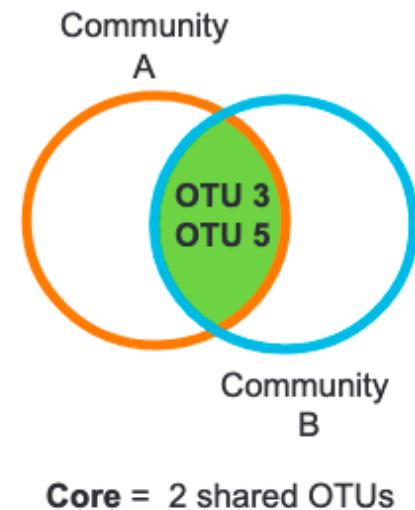
(Shade et al., 2011)

# Core microbiota

## D. Persistence:

OTUs shared over time within and across communities

List of observed OTUs	Time series community A			Time series community B			Shared persistence A & B
	time 1	time 2	time 3	time 1	time 2	time 3	
	OTU 1	0.4	0	0	0	0	
OTU 2	0	0	0	0.2	0.2	0	
OTU 3	0.1	0.1	0.2	0.1	0.1	0.1	X ●
OTU 4	0.2	0	0	0.2	0.1	0.1	
OTU 5	0.3	0.9	0.8	0.7	0.6	0.8	X ●

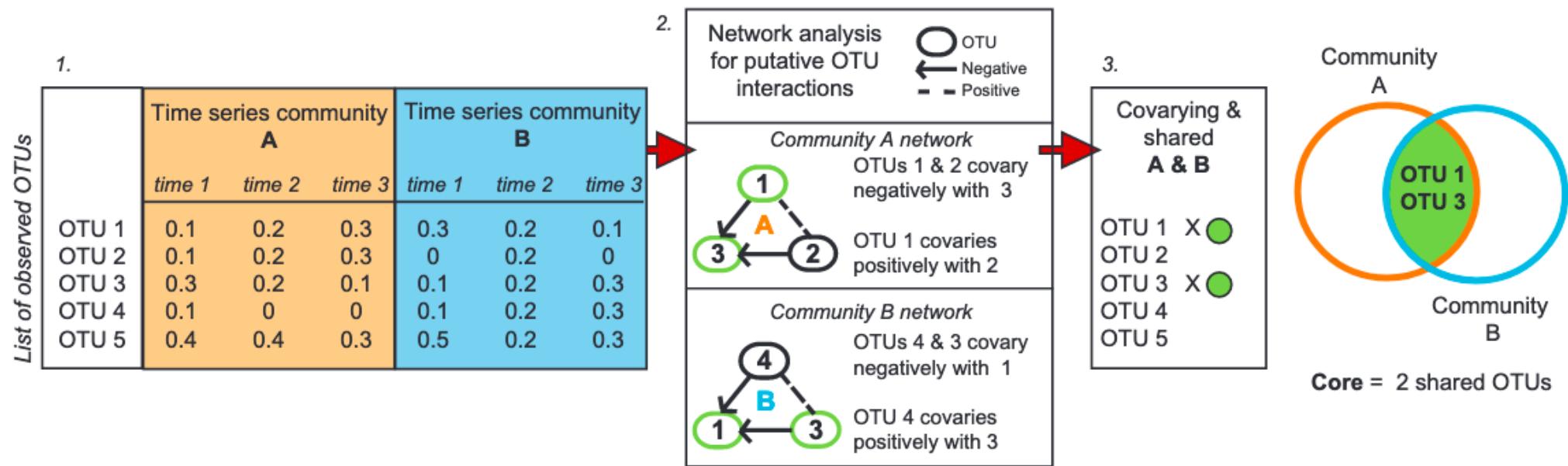


(Shade et al., 2011)

# Core microbiota

## E. Connectivity:

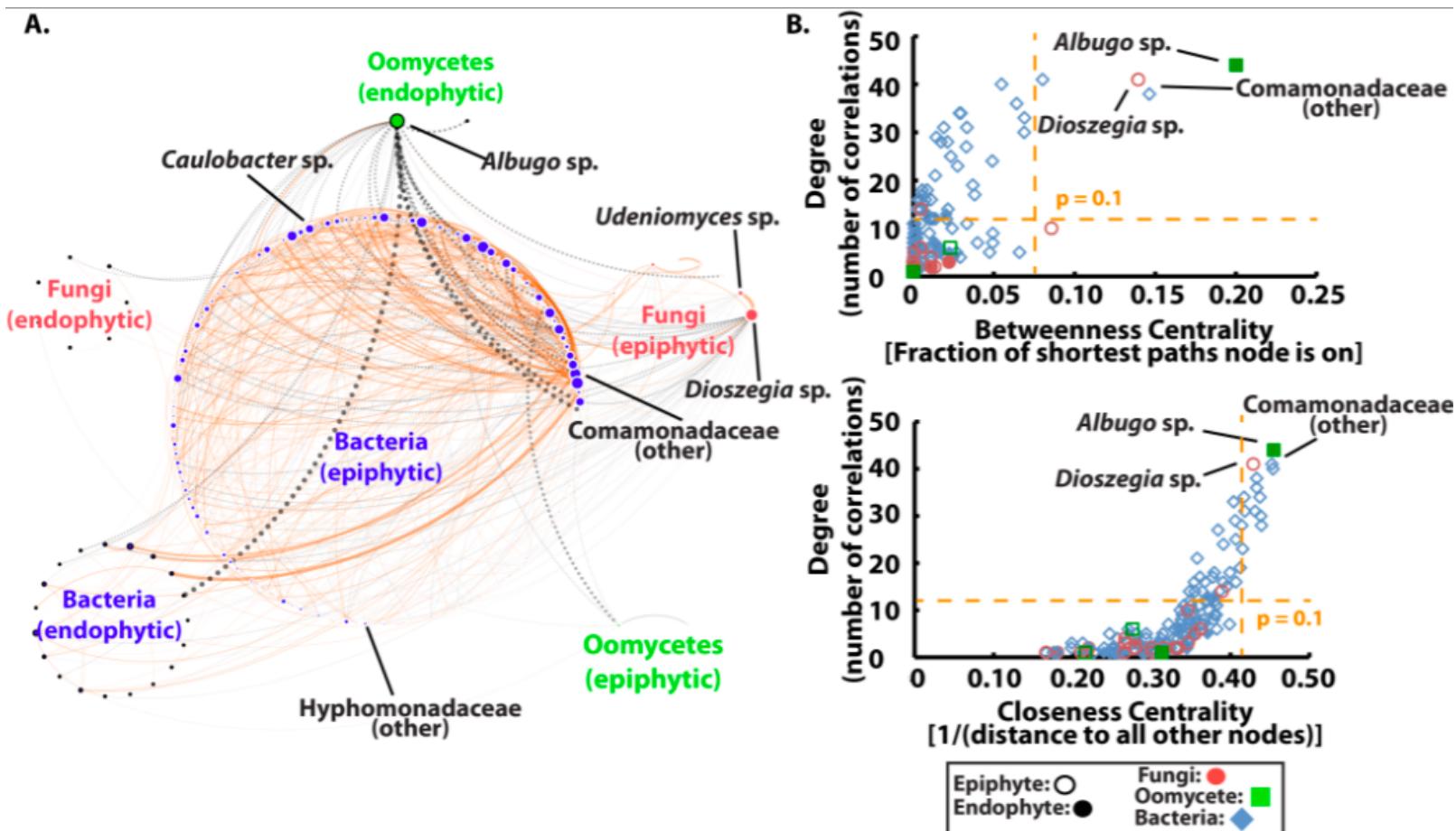
OTUs co-varying within a community and shared across communities



(Shade et al., 2011)

# Hub microbiota

# Hub microbiota



(Agler et al., 2016)

# Agenda

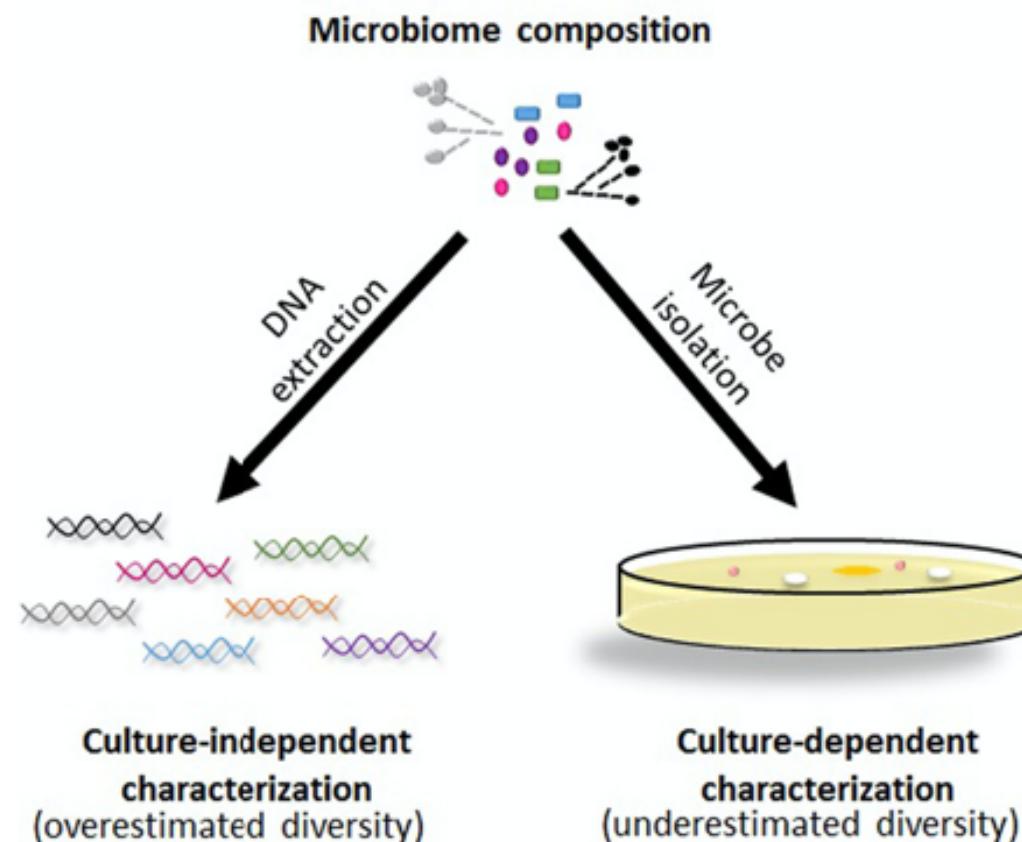
- How do you study the microbiota composition?
- What can the microbiota potentially do?
- What are they doing?

How do you study the microbiota composition?

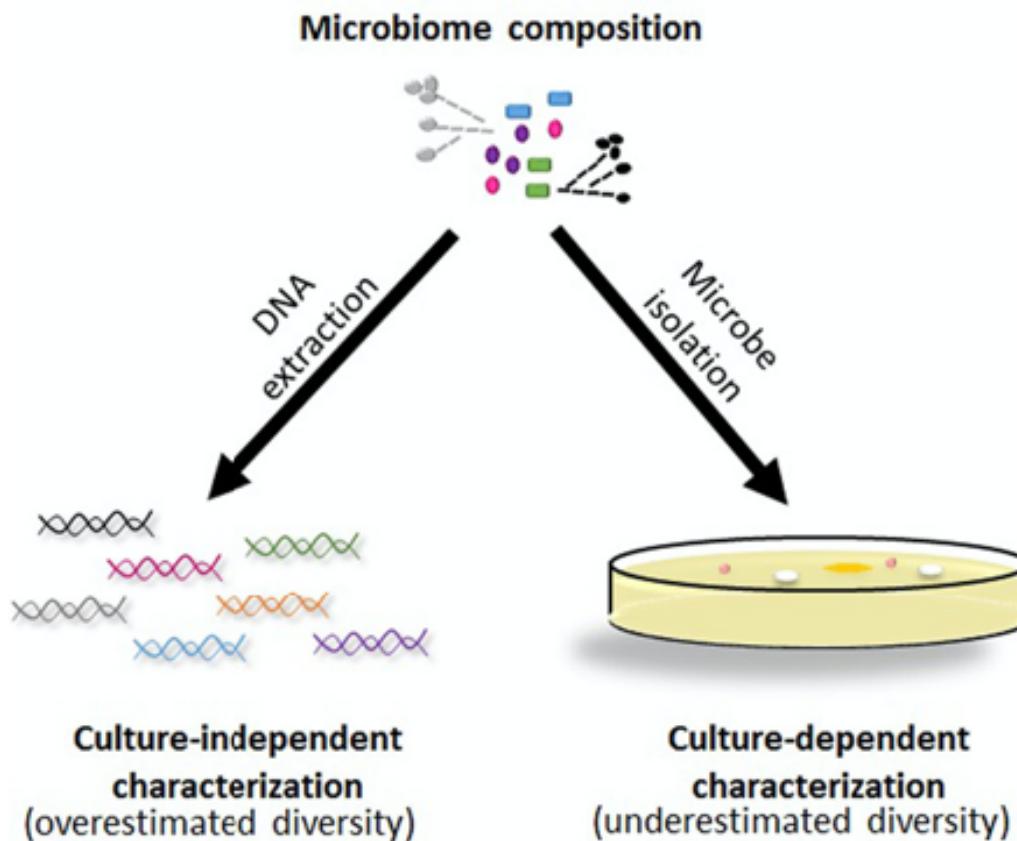
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# How do you study the microbiota composition?

**There are two major approaches to assess the microbiota composition**



# How do you study the microbiota composition?



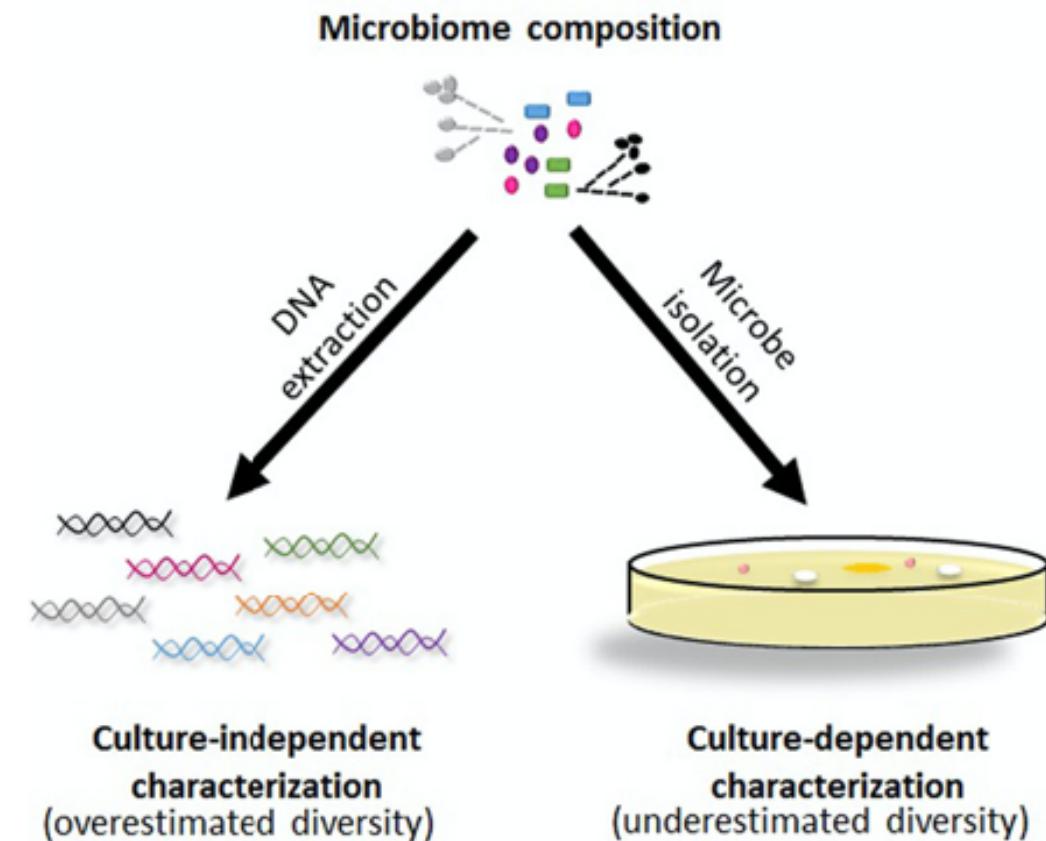
## Culture dependent

- + This approach isolates individual microbes
- ✖ The community diversity estimate is limited

# How do you study the microbiota composition?

## Culture independent

- + The ability to identify and quantify community members, even down to extremely rare taxa
- + Insights beyond the information provided by individual microbes
- ✖ This approach does not isolate individual microbes



# How do you study the microbiota composition?

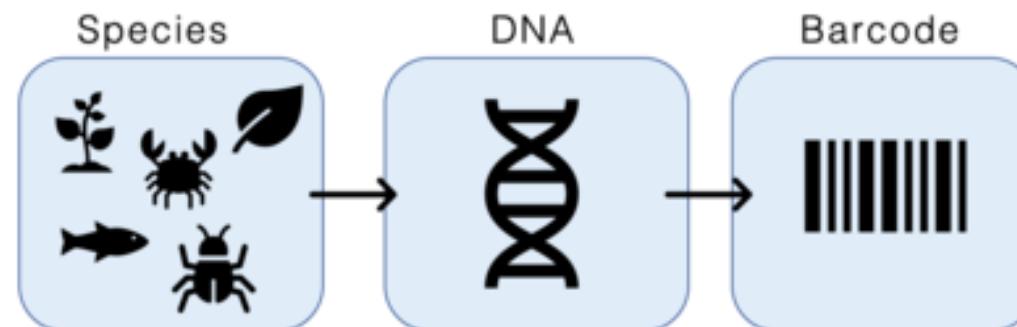
Microbial DNA metabarcoding

**DNA metabarcoding** is a method of **DNA barcoding** that uses universal genetic markers to identify DNA of a mixture of organisms

# How do you study the microbiota composition?

## Microbial DNA metabarcoding

- **DNA barcoding** is a method of species identification using a short section of DNA from a specific gene
- An individual sequence can be used to uniquely identify an organism by comparison with a reference library of classified DNA sequences

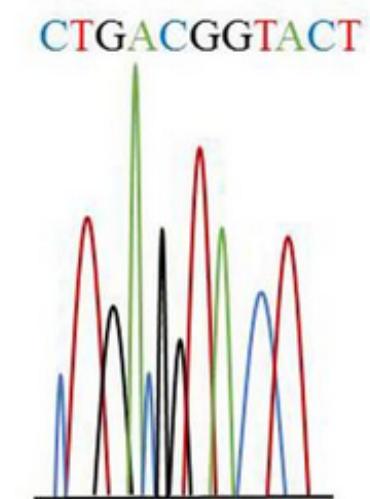


# How do you study the microbiota composition?

## Microbial DNA metabarcoding

### Marker selection

- Markers used for DNA barcoding are called barcodes and their selection is crucial
- Ideally, one gene sequence would be used for developing universal PCR primers for all taxonomic group
- However, no such gene region has been found yet!
- Different gene regions are used to identify the different organismal groups using barcoding



**Sequencing of DNA barcode**

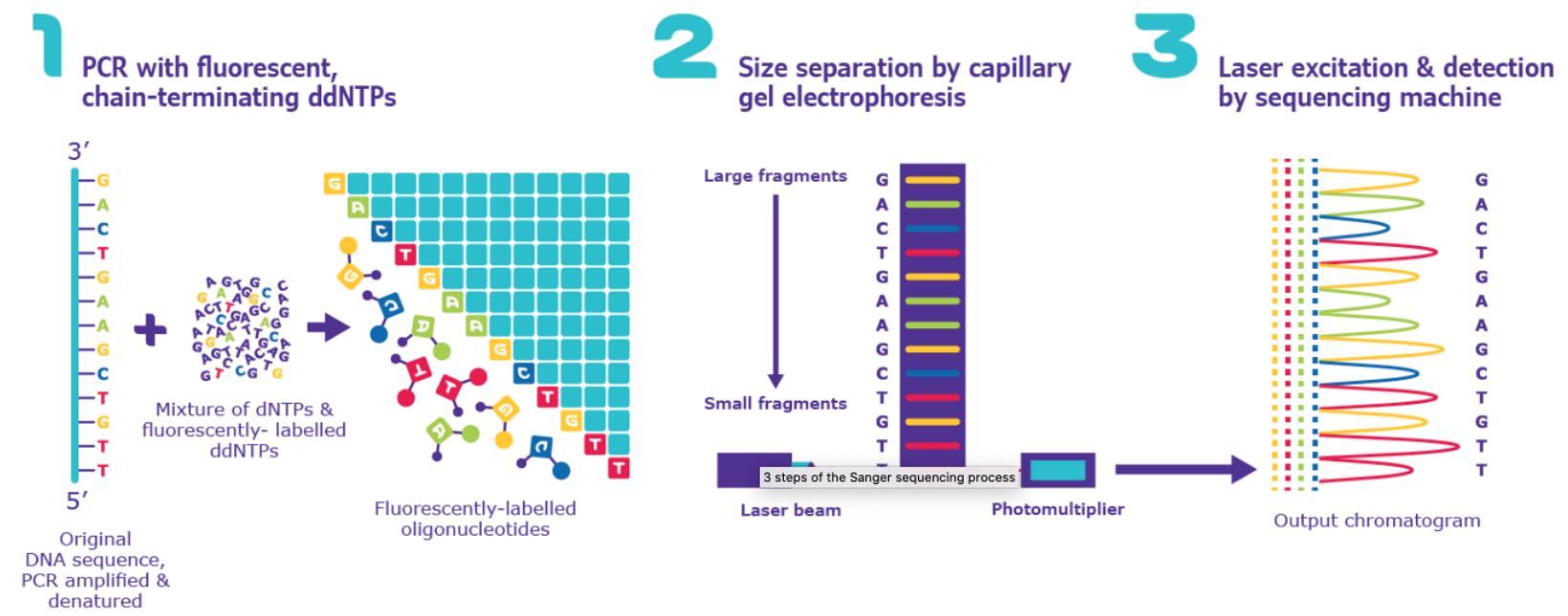
(Mosa et al., 2019)

# How do you study the microbiota composition?

## Sequencing

### Sanger Sequencing

Sanger sequencing, also known as the "chain termination method", is a method for determining the nucleotide sequence of DNA. The method was developed by two time Nobel Laureate Frederick Sanger and his colleagues in 1977, hence the name the Sanger Sequence.



# How do you study the microbiota composition?

## Microbial DNA metabarcoding

### Marker selection

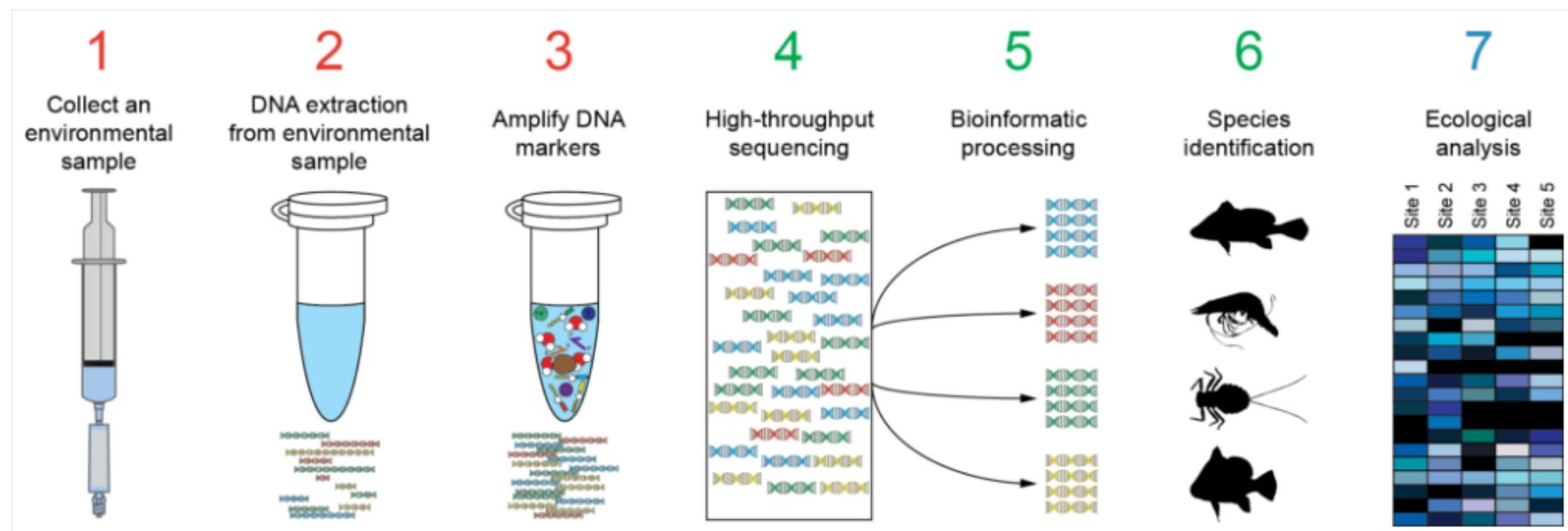
Organism	Region
animals	cytochrome c oxidase I
fungi	internal transcribed spacer (ITS) rRNA
plants	RuBisCO
prokaryotes	16S rRNA
microbial eukaryotes	18S rRNA

When barcoding is used to identify organisms from a sample containing DNA from more than one organism, the term DNA metabarcoding is used

# How do you study the microbiota composition?

## Microbial DNA metabarcoding

### Metabarcoding process



Metabarcoding workflow. Source: <http://www.naturemetrics.co.uk>

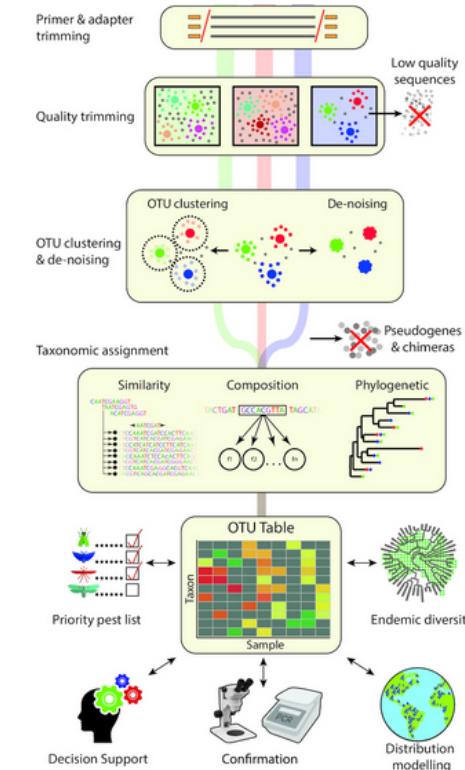
# How do you study the microbiota composition?

## Microbial DNA metabarcoding

### Bioinformatic analysis

(Jesse et al., 2018)

- After sequencing, raw data must be processed (**quality and cleaning step**)
- Samples are clustered in operational taxonomic unit based on identity threshold (**OTU formation**)
- Using "the Basic Local Alignment Search Tool (BLAST)" regions of similarity between sequences and reference databases are identified (**annotation step**)
- Microbiota composition definition (**richness and diversity step**)

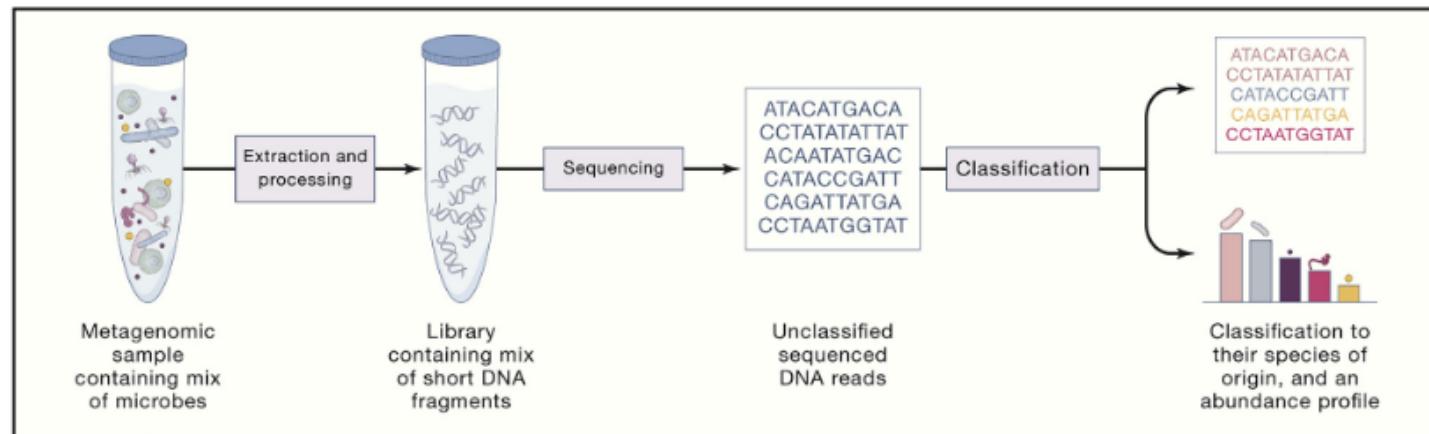


# What can the microbiota potentially do?

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# What can the microbiota potentially do?

## Metagenomics

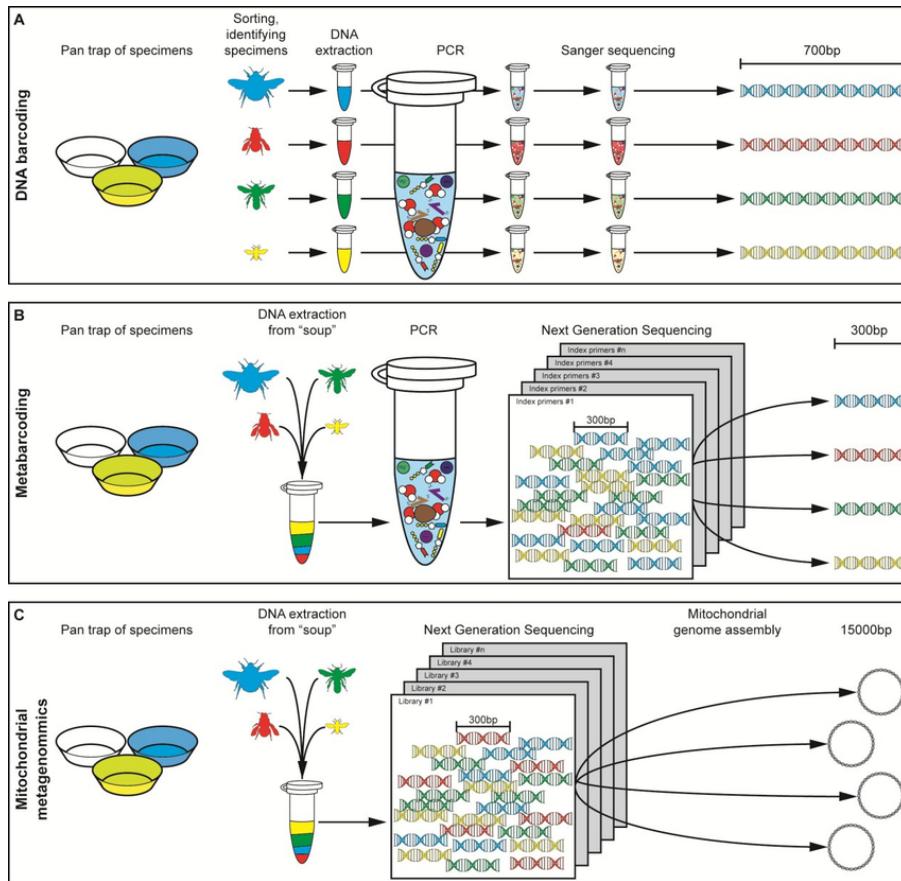


(Ye, Siddle, Park, and Sabeti, 2019)

- **Metagenomics** is the study of genetic material recovered directly from environmental samples
- The collection of sequenced genes from the environment could be analyzed as a **single genome**
- Metagenomics **does not require the isolation** and lab cultivation of individual species

# What can the microbiota potentially do?

## Metagenomics vs Metabarcoding



- Metagenomics does not need the marker selection step
- Metagenomics sequences all the genes present in the sample from each organism
- Metagenomics can help to reconstruct large fragments or even complete genomes from organisms in a community
- Metagenomics allows the characterization of a large number of coding and non-coding sequences that can be used to decipher the microbial diversity or to understand its metabolic potential

# What can the microbiota potentially do?

Comparison of high-throughput sequencing methods						
Source: Wikipedia						
METHOD	TECH	READ LENGTH	ACCURACY SINGLE READ NOT CONSENSUS	READS PER RUN	TIME PER RUN	COST PER 1 BILLION BASES IN US
Chain termination	Sanger sequencing	400 to 900 bp	99.9%	N/A	20 minutes to 3 hours	\$2,400,000
Pyrosequencing	454	700 bp	99.90%	1 million	24 hours	\$10,000
Ion semiconductor	Ion Torrent sequencing	up to 600 bp	99.60%	up to 80 million	2 hours	\$66.8-\$950
Single-molecule real-time sequencing	Pacific Biosciences	On average 30,000 bp	87.00%	up to 4 millions	30 minutes to 20 hours	\$7.2-\$43.3
Nanopore Sequencing	Nanopore	up to 2,272,580 bp reported	~92–97% single read	dependent on read length selected by user	1 min to 48 hrs	\$7–100
Sequencing by synthesis	Illumina	MiniSeq: 75–300 bp	99.90%	MiniSeq: 1–25 Million	1 to 11 days, depending upon sequencer and specified read length	\$5 to \$150
Sequencing by synthesis	Illumina	NextSeq: 75–300 bp	99.90%	NextSeq: 130-00 Million	1 to 11 days, depending upon sequencer and specified read length	\$5 to \$150
Sequencing by synthesis	Illumina	MiSeq: 50–600 bp	99.90%	MiSeq: 1–25 Million	1 to 11 days, depending upon sequencer and specified read length	\$5 to \$150
Sequencing by synthesis	Illumina	HiSeq 2500: 50–500 bp	99.90%	HiSeq 2500: 300 million – 2 billion	1 to 11 days, depending upon sequencer and specified read length	\$5 to \$150
Sequencing by synthesis	Illumina	HiSeq 3/4000: 50–300 bp	99.90%	HiSeq 3/4000 2.5 billion	1 to 11 days, depending upon sequencer and specified read length	\$5 to \$150
Sequencing by synthesis	Illumina	HiSeq X: 300 bp	99.90%	HiSeq X: 3 billion	1 to 11 days, depending upon sequencer and specified read length	\$5 to \$150

# What can the microbiota potentially do?

Illumina Sequencing by Synthesis



<https://bit.ly/2IGGiJE>

# What can the microbiota potentially do?

Introduction to nanopore sequencing



<https://bit.ly/3FFx1dM>

# What can the microbiota potentially do?

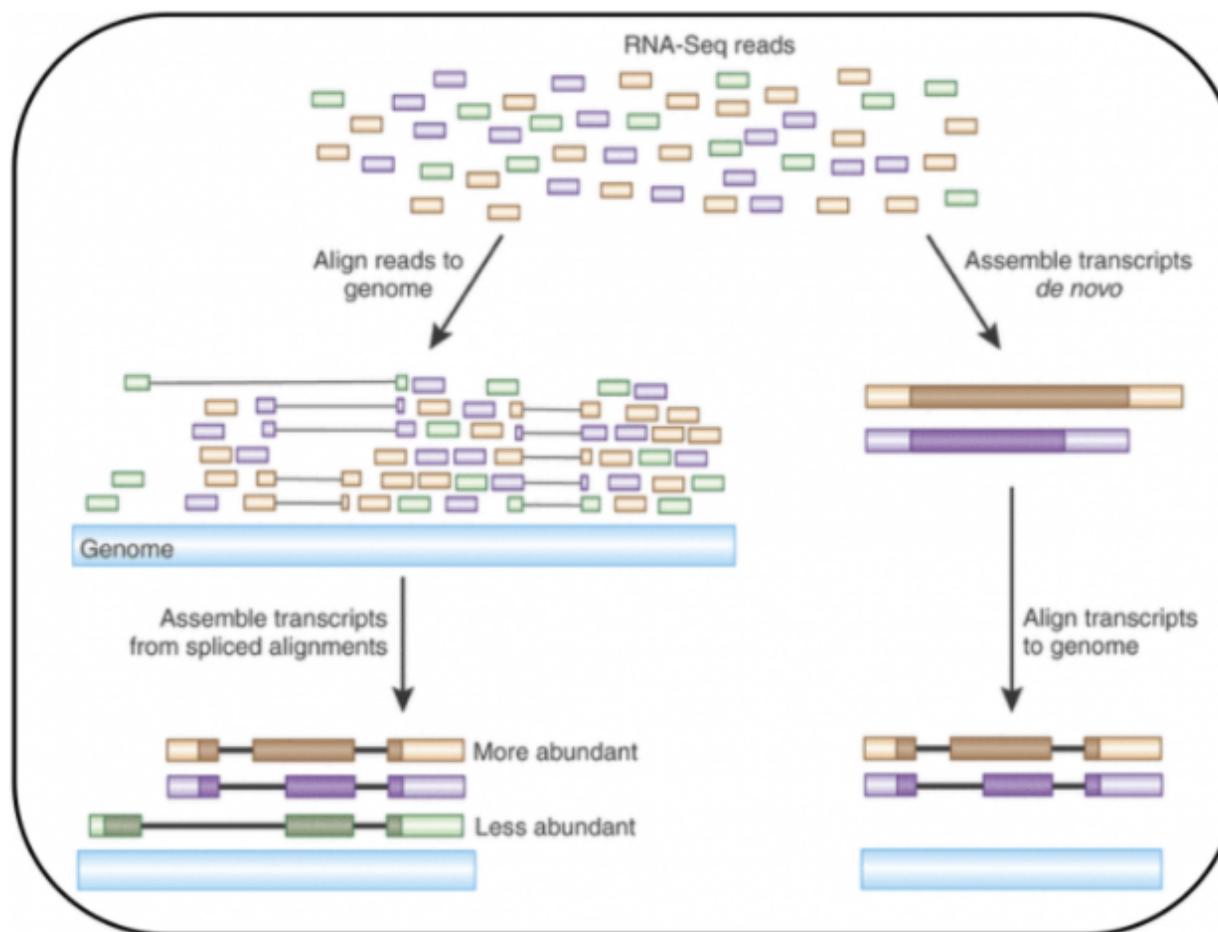
PacBio Sequencing – How it Works



<https://bit.ly/3FFx1dM>

# What can the microbiota potentially do?

## Genomes assembly

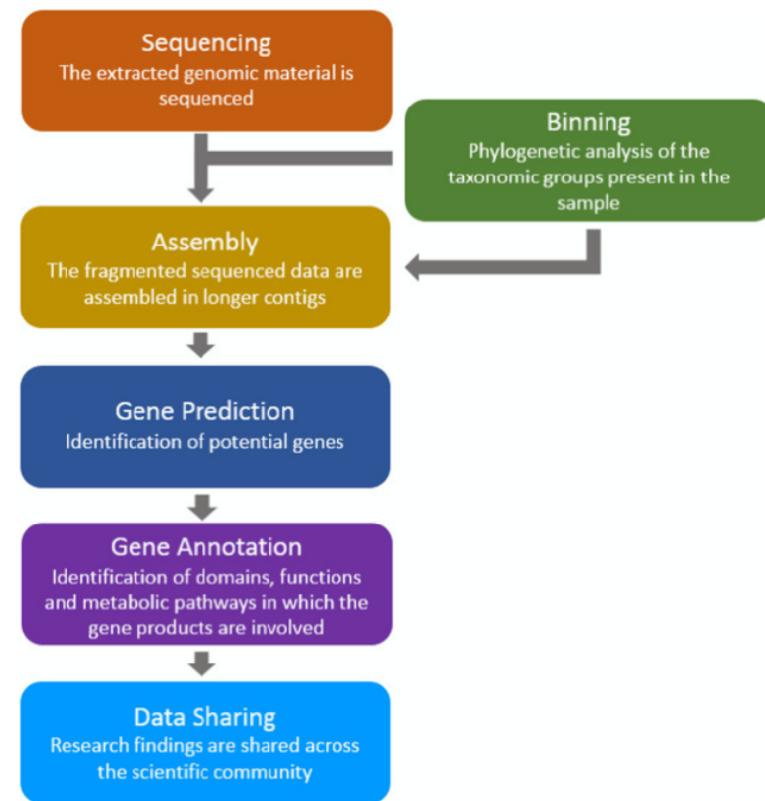


# What can the microbiota potentially do?

## Functional Metagenomics Analysis

Workflow:

- Binning
- Metagenomic assembly
- Gene prediction
- Gene annotation
- Reconstruction of metabolic pathways (achieved from enzyme-coding genes)



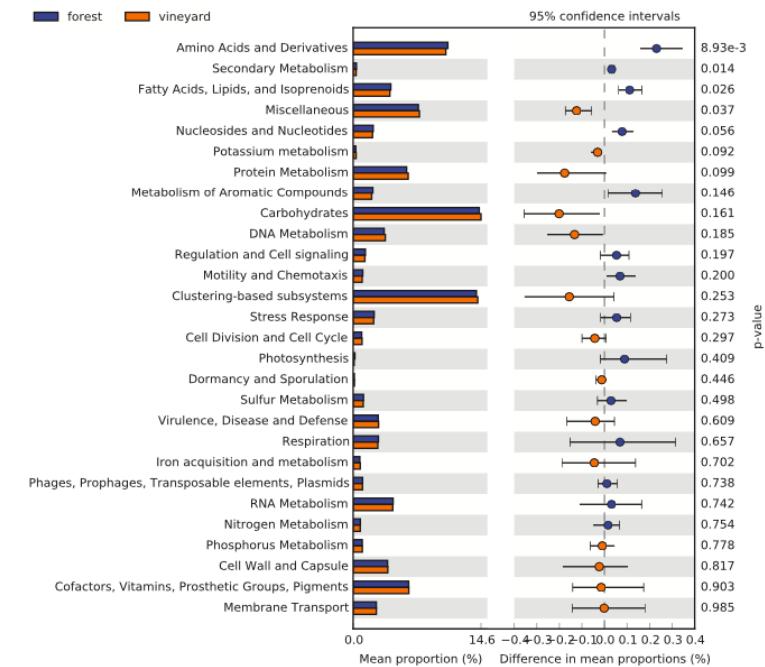
(Roumpeka, Wallace, Escalettes, Fotheringham, and Watson, 2017)

# What can the microbiota potentially do?

## Functional Metagenomics Analysis

Workflow:

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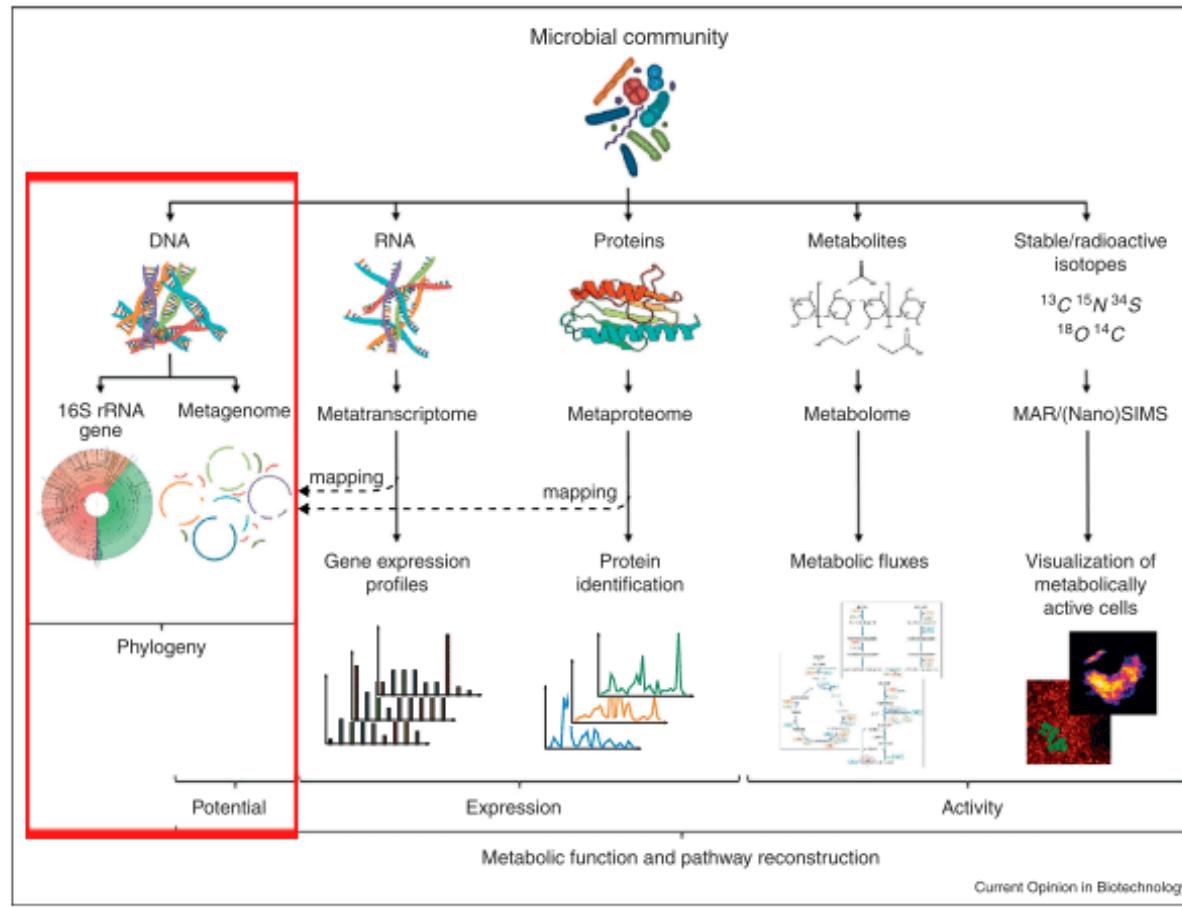


**Figure 4** Functional categories found in soil microbial communities. Bar plot showing the mean proportion (%) of functional categories found in soil microbial communities based on the subsystem database. Points indicate the differences between forest and vineyard soils (blue and orange bars, respectively), and the values at the right show the P-values were derived from a White's non-parametric t-test (White, Nagarajan & Pop, 2009).

# What are they doing?

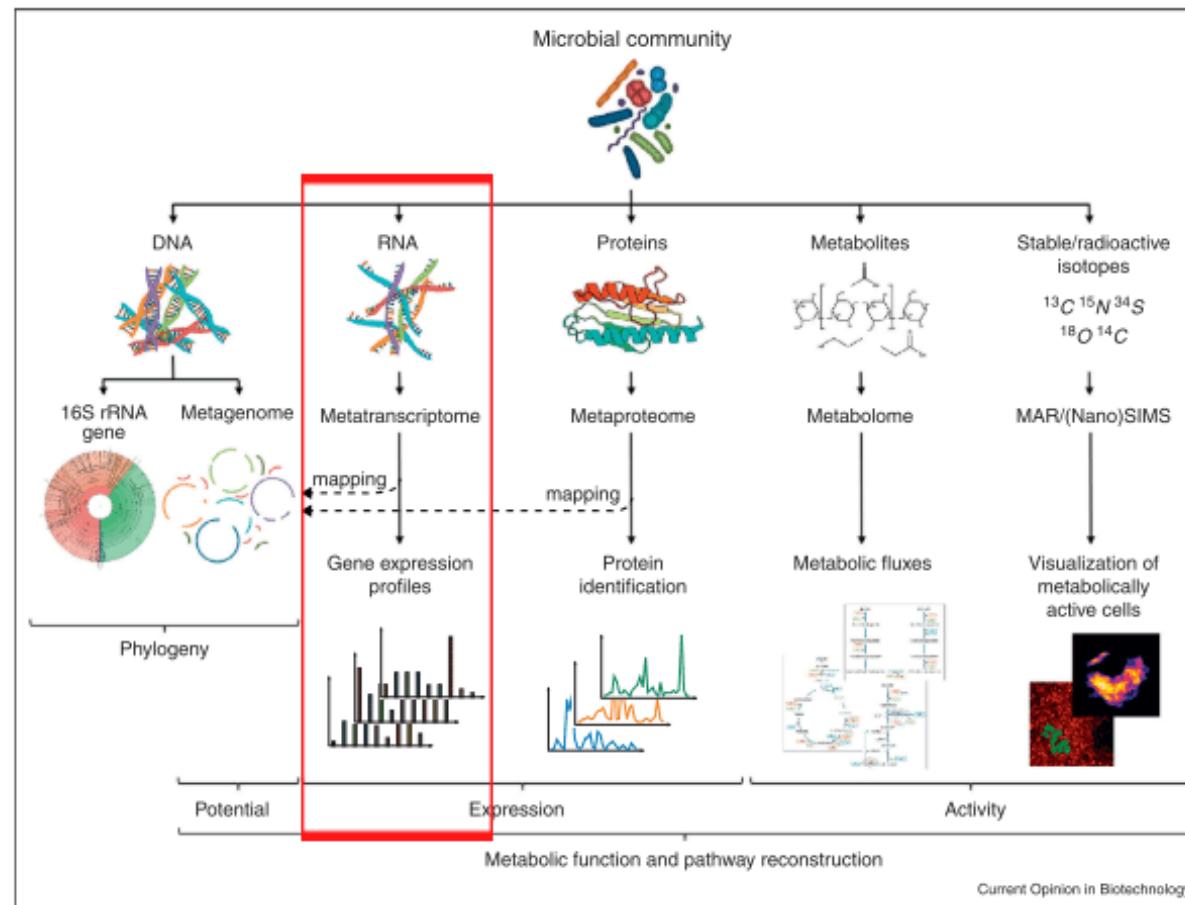
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# What are they doing?



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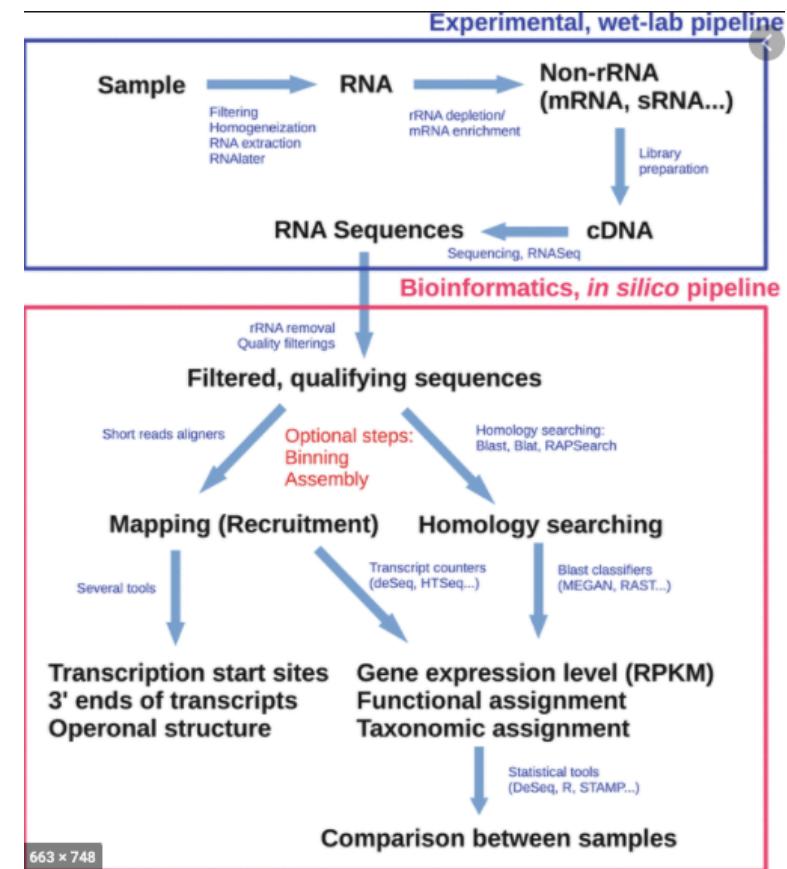
## Metatranscriptomics



# What are they doing?

## Metatranscriptomics

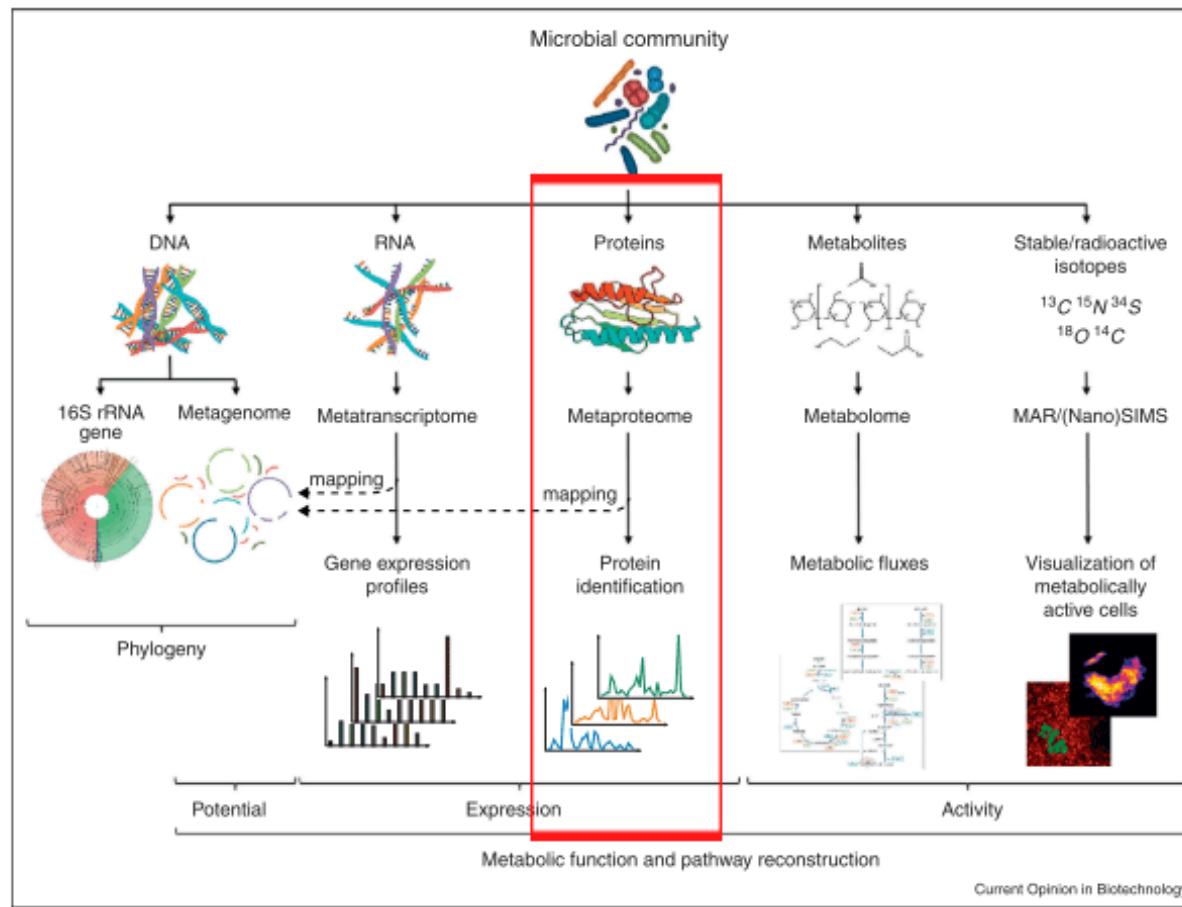
- Metatranscriptomics is the science that studies gene expression of microbes within natural environments
- It allows to obtain whole gene expression profiling of complex microbial communities
- **+ The advantage of metatranscriptomics, over metagenomics, is that it can provide information about differences in the active functions of microbial communities**
- The overview of the gene expression in a given sample is obtained by capturing the total mRNA of the microbiome and by performing a whole metatranscriptomics shotgun sequencing.



(Pérez-Pantoja and Tamames, 2015)

# What are they doing?

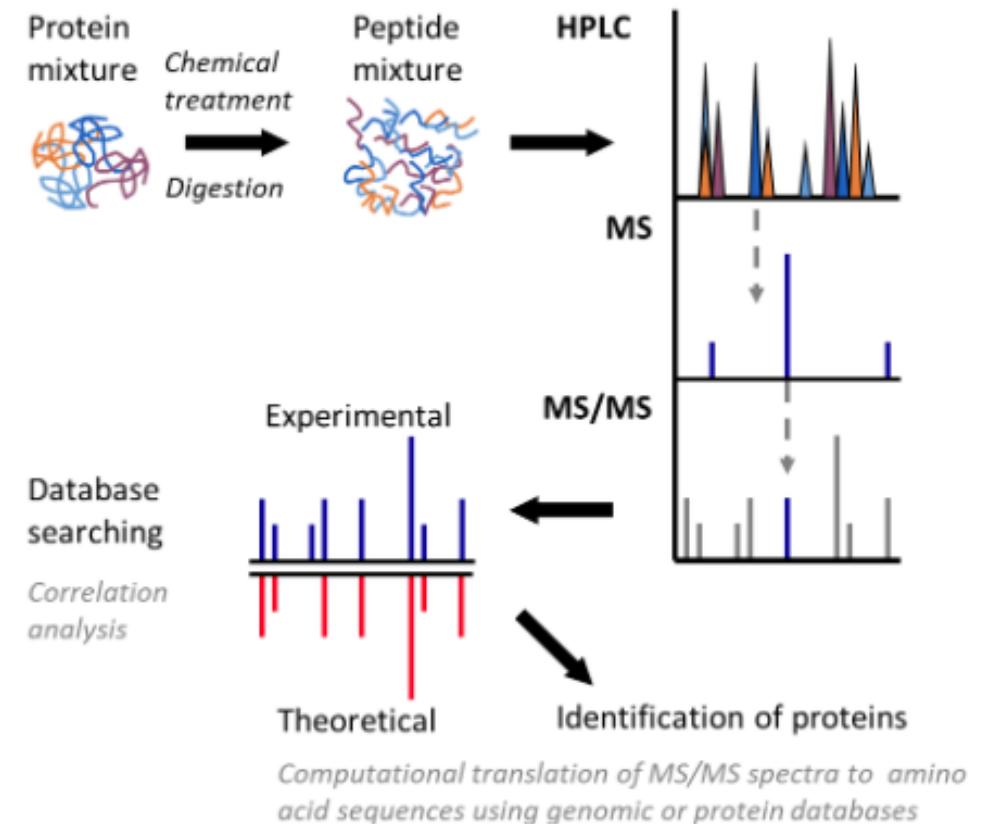
## Metaproteomics



# What are they doing?

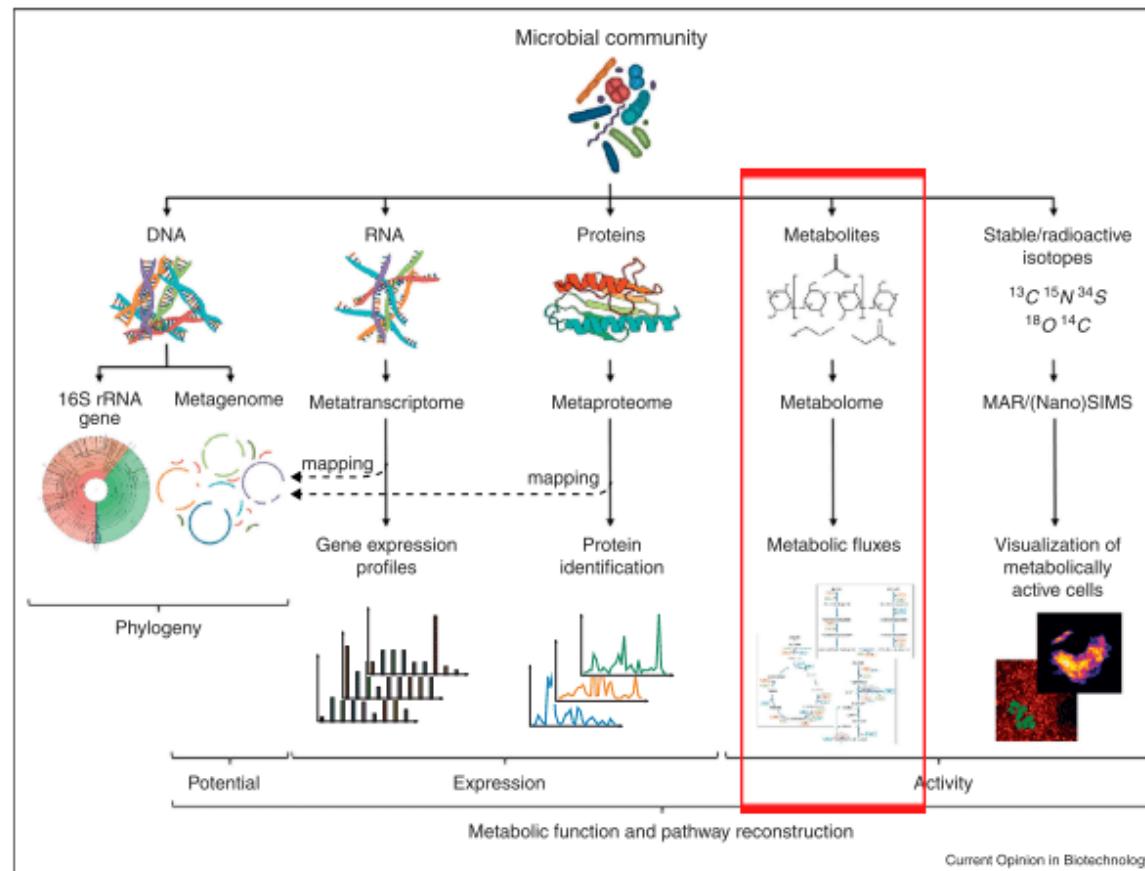
## Metaproteomics

- Metaproteomics is the study of all protein samples recovered directly from environmental sources
- Metaproteomics have rapidly increased in recent years due to many technological advances in mass spectrometry (MS)
- **+ Metaproteomics** assess the “expressed” metabolism and physiology of microbial community members, but also allows quantification of per-species biomass to determine community structure
- **+ Proteomics** quantifies the real proteins expression level, while mRNA may be degraded or translated inefficiently, resulting in an incorrect estimation of protein expression level



# What are they doing?

## Metabolomics

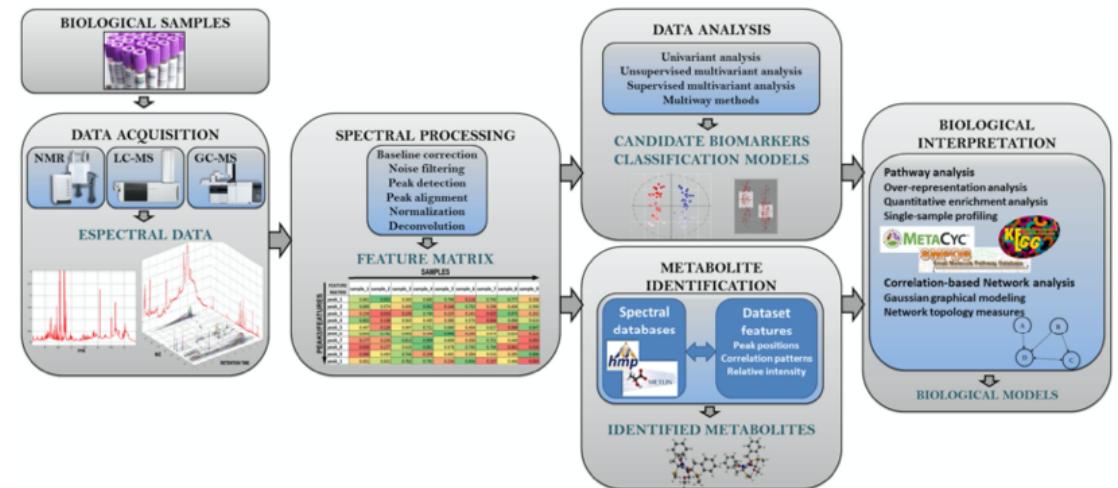


(Vanwonterghem, Jensen, Ho, et al., 2014)

# What are they doing?

## Metabolomics

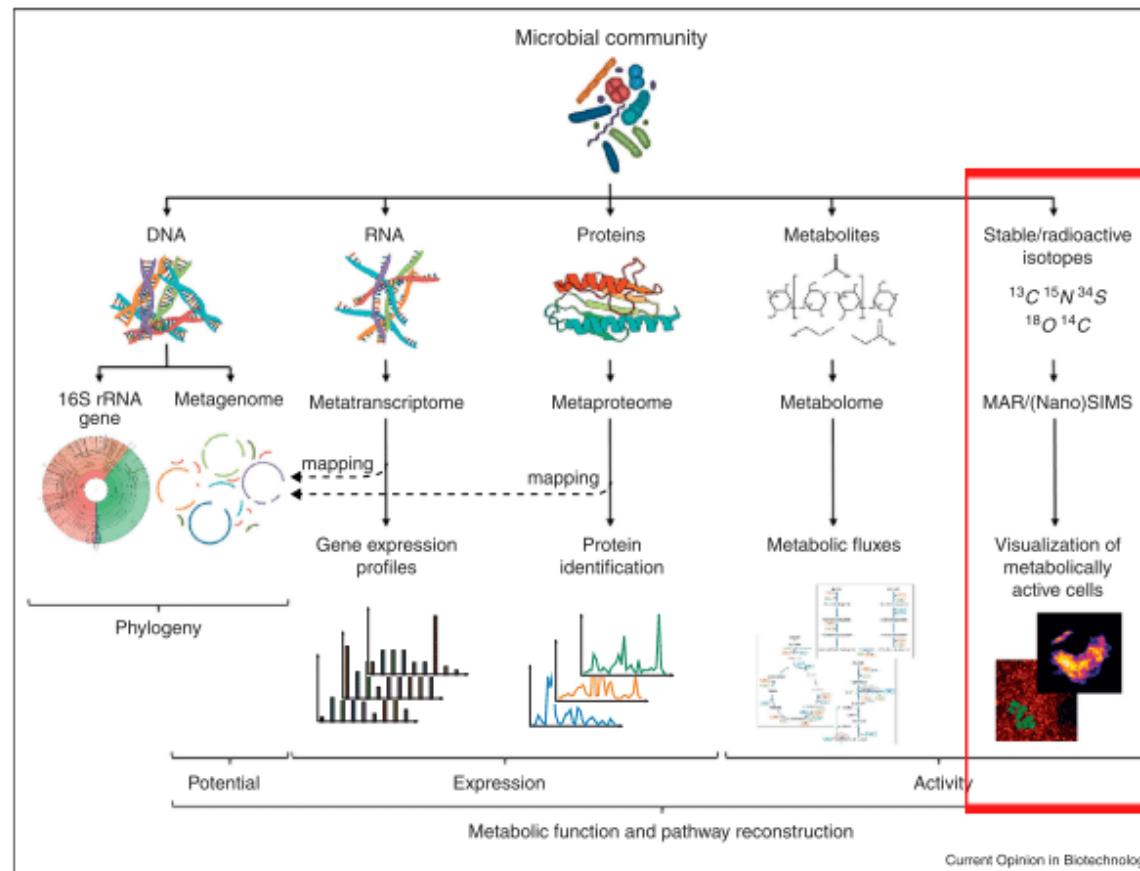
- Metabolomics is the study of the complete set of metabolites composition (the metabolome) of a cell type, tissue, or biological fluid
- **+** The metabolites (<1.5 kDa) are the intermediates or end products of multiple enzymatic reactions and therefore are the most informative proxies of the biochemical activity of an organism
- **✗** The metabolome consists of multiple compounds.  
Determine the entire set of metabolites is extremely difficult, further complicating the analyses is the dynamic nature of these metabolites.



(Alonso, Marsal, and Julià, 2015)

# What are they doing?

Stable/radioactive isotopes

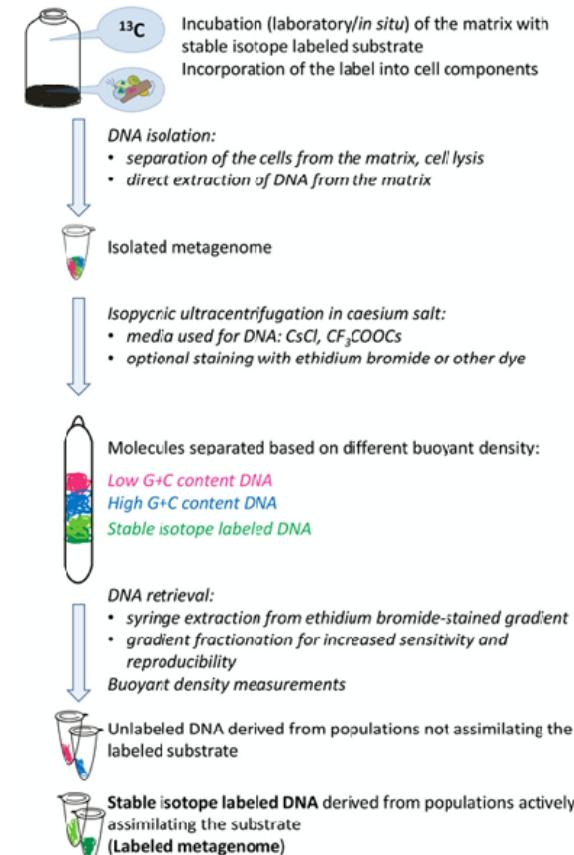


(Vanwonterghem, Jensen, Ho, et al., 2014)

# What are they doing?

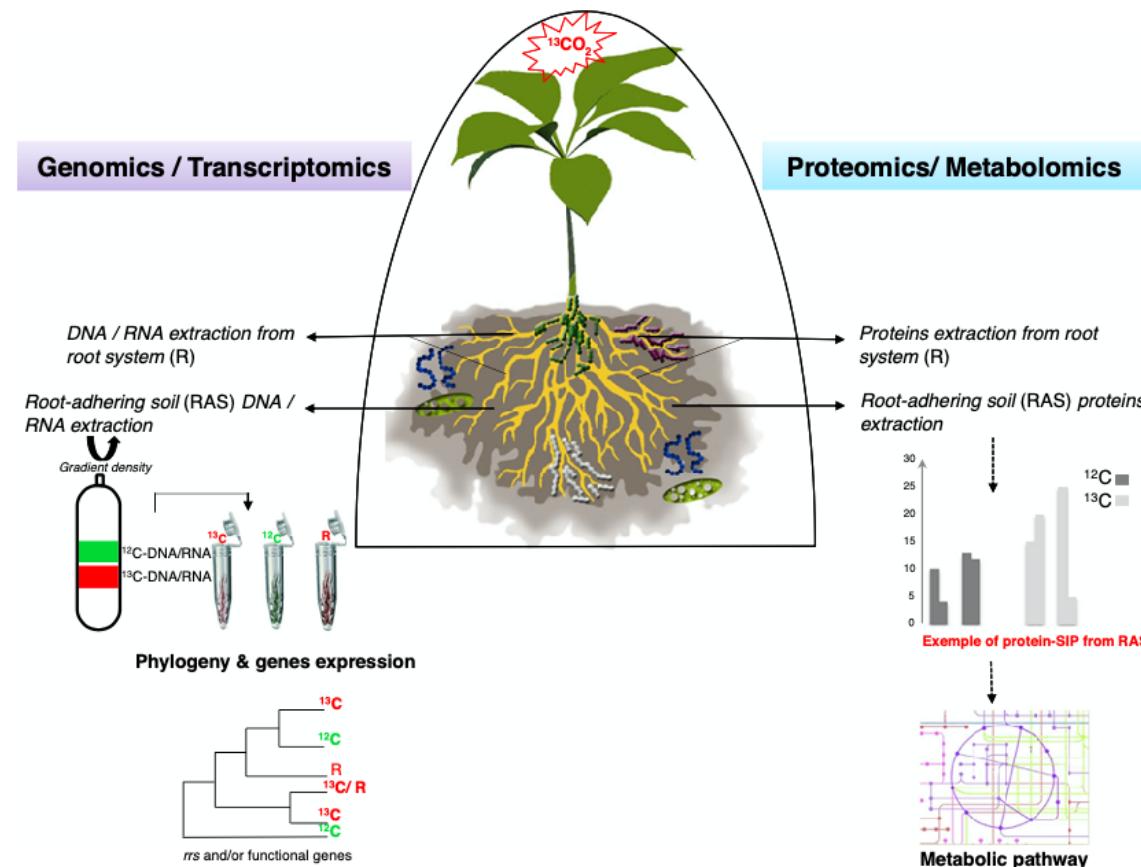
## Stable/radioactive isotopes

- Stable-isotope probing (SIP) is a technique in microbial ecology for tracing fluxes of nutrients in biogeochemical cycling by microorganisms using heavy stable isotopes
- + SIP is an approach that can identify microbial populations with a defined function
- + The use of either DNA-SIP, RNA-SIP or protein-SIP can enable both the phylogenetic identification and the key metabolic genes/proteins
- ✗ A major challenge associated with SIP is a very limited availability and high cost of labeled substrates. SIP is also very labor-intensive and low-throughput using current techniques

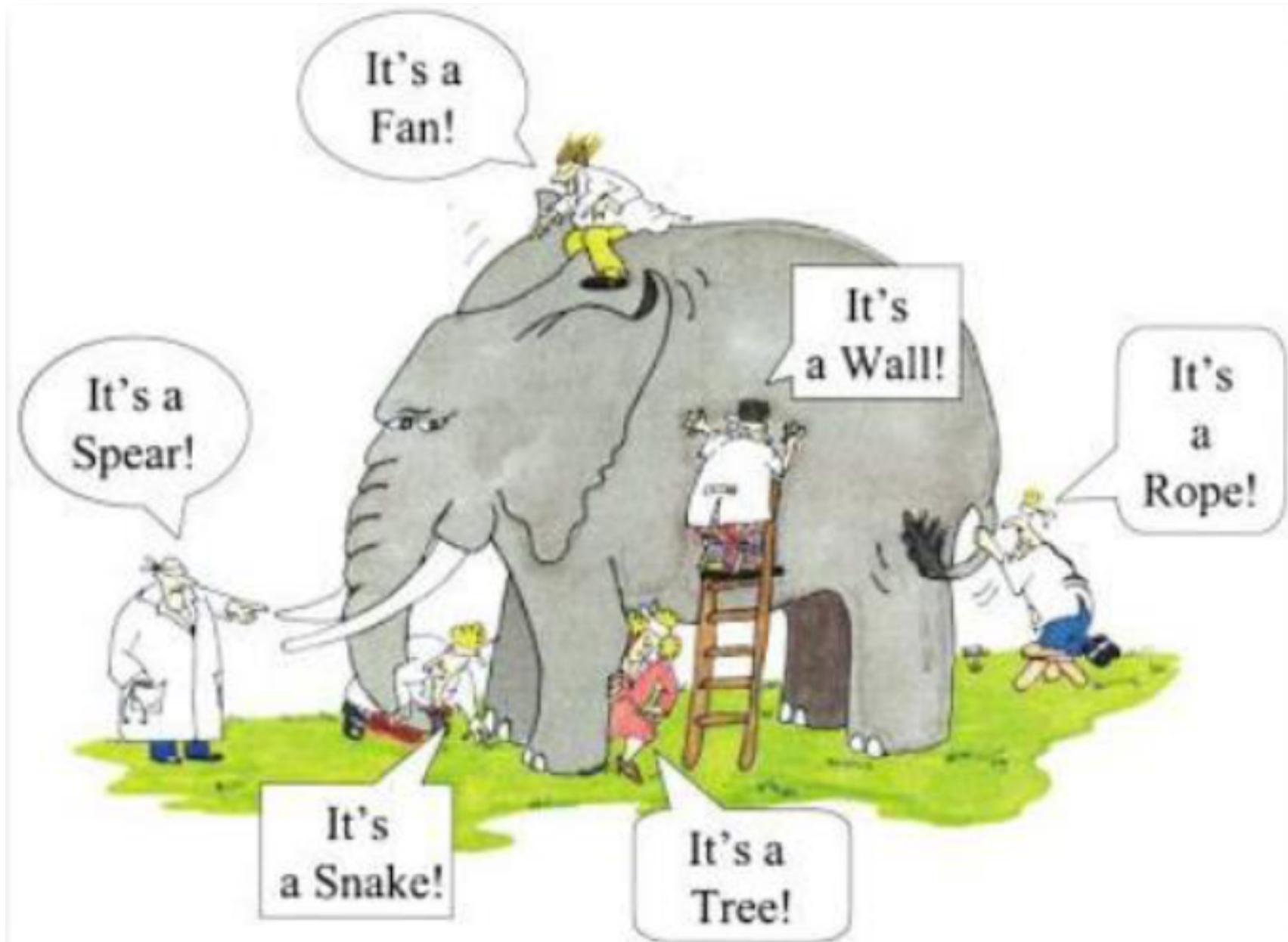


# What are they doing?

Stable/radioactive isotopes

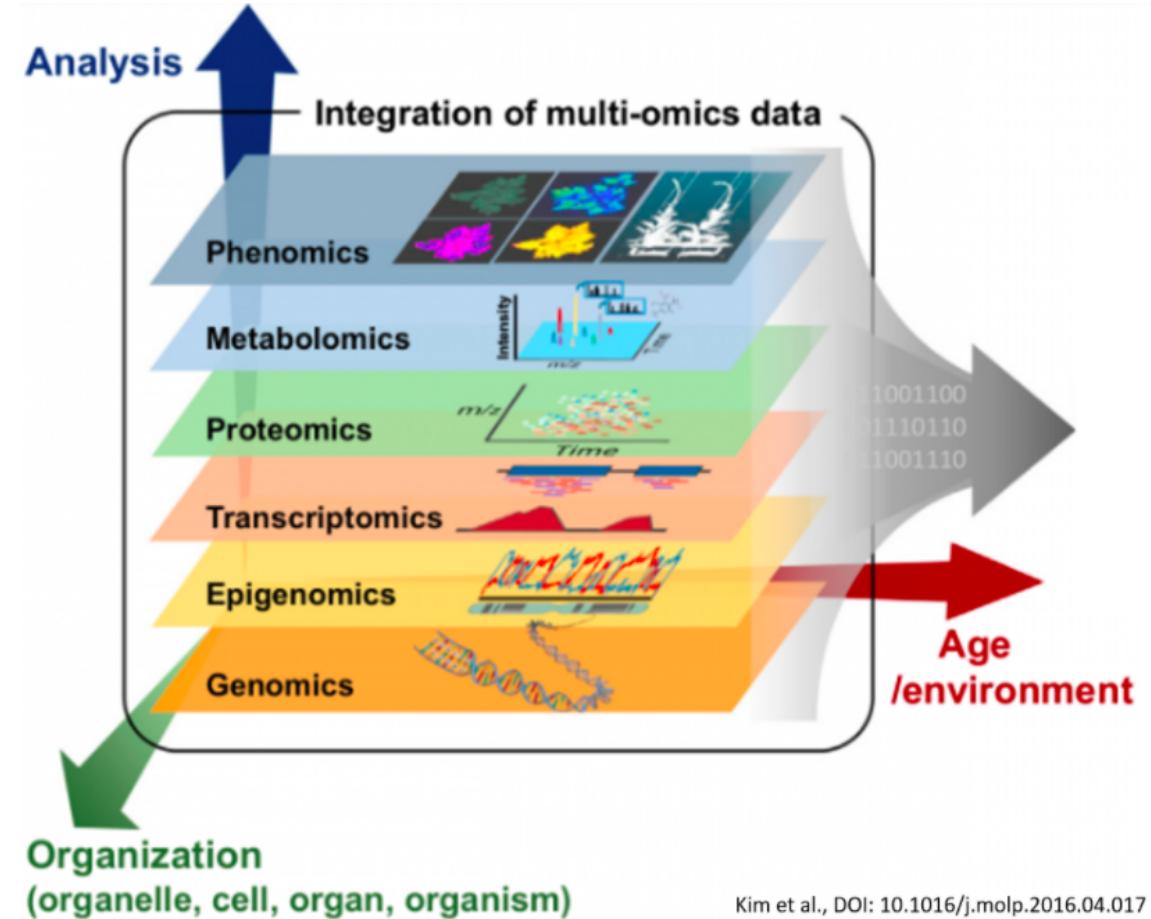


(el Zahar Haichar, Heulin, Guyonnet, et al., 2016)



# System biology

- Systems biology studies biological systems by systematically perturbing them
- Monitoring the gene, protein, metabolite, and informational pathway responses
- Integrating these data
- Formulating mathematical models that describe the structure of the system
- Predict its response to individual perturbations

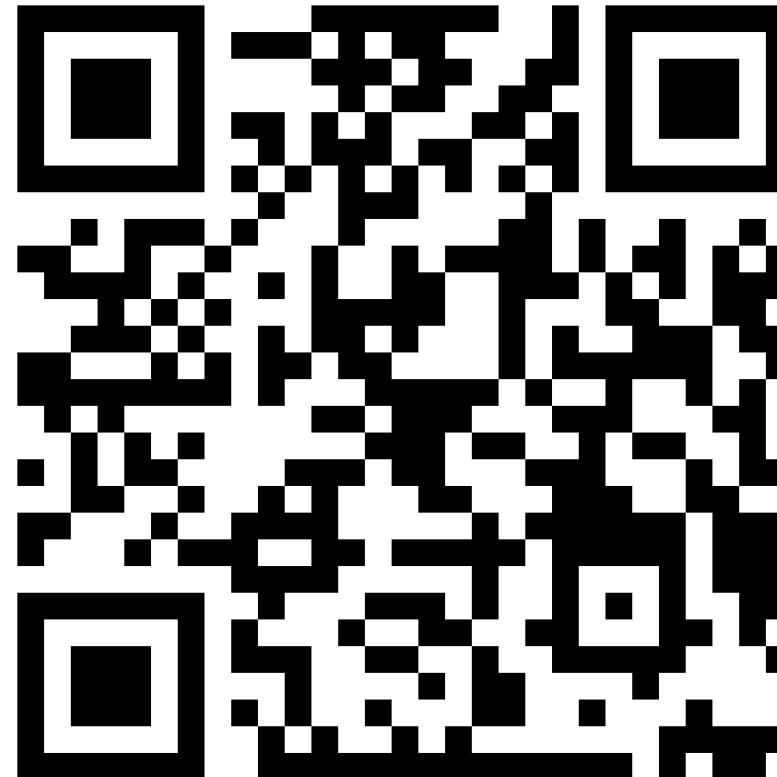


# Questions about the lesson



Slack (<https://bit.ly/3upNjCt>) -  marco.chiapello@unito.it

# Feedbacks



<https://bit.ly/2YMIzD3>