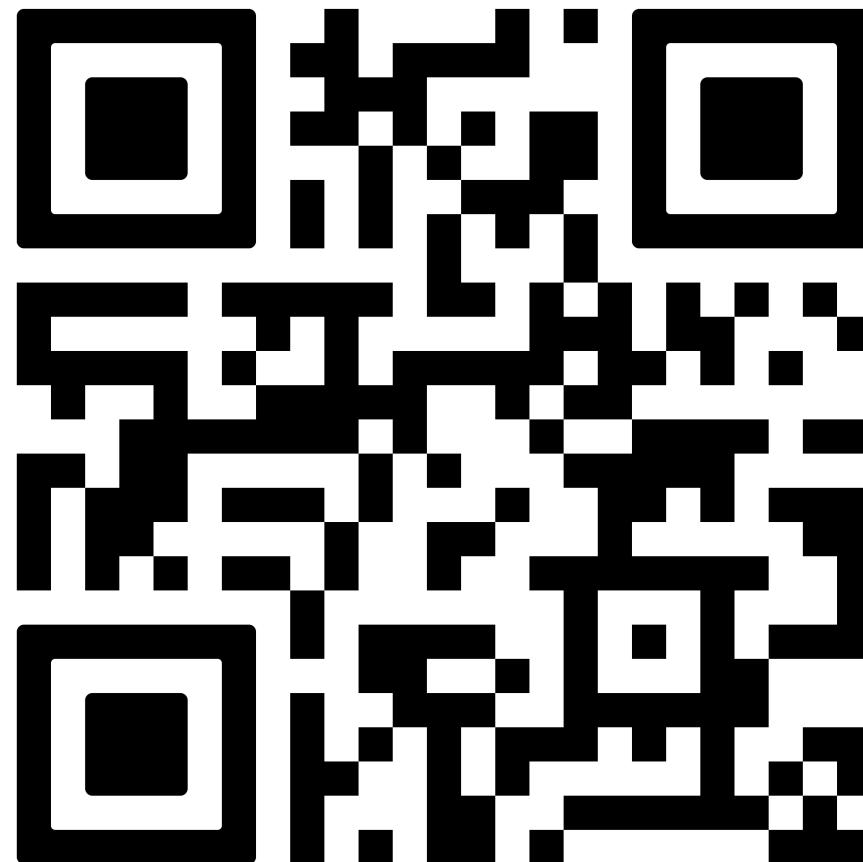


# MICROBIOMA E MICROBIOTA

Marco Chiapello

2020-11-17

# Last lesson check



# Il microbiota vegetale varia?

Opzioni	Risposte
Si, ma solo tra la composizione del microbiota presente nella rizosfera e quello dell'endosfera	1 <span style="color: red;">✖</span>
Si, varia nel tempo. In diversi momenti della vita della pianta la composizione del microbiota muta	15 <span style="color: green;">✓</span>

Qual è la forza selettiva maggiore che da forma al microbiota associato alle piante?

Opzioni	Risposte
Il microbiota	3
L'evoluzione	6
La pianta	7

# Per olobiote si intende...

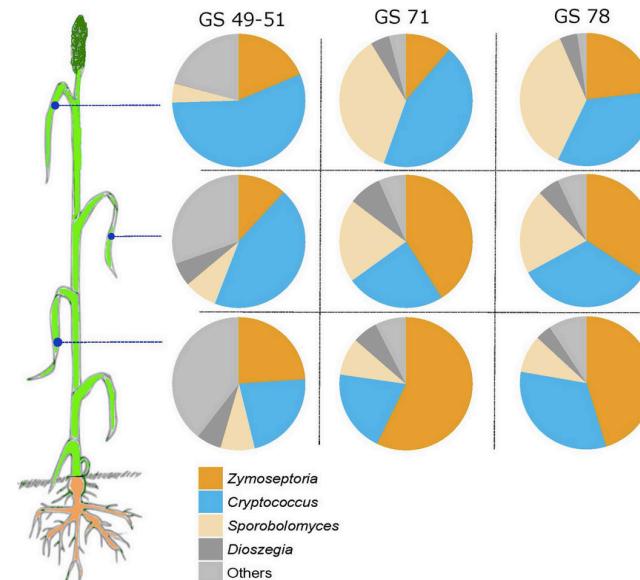
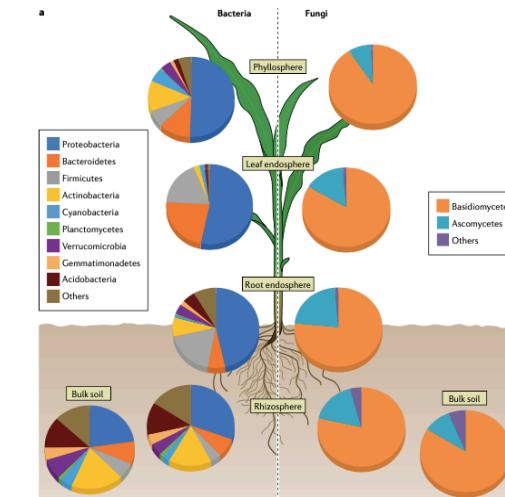
Opzioni	Risposte
L'insieme del genoma dell'organismo ospite e del genoma dei microrganismi che lo abitano	7 <span style="color: red;">✗</span>
Un organismo vivente alla luce della convivenza con il proprio microbiota	5 <span style="color: green;">✓</span>
membri del microbiota che sono presenti in tutte, o quasi, le comunità associate ad una determinato ospite	4 <span style="color: red;">✗</span>

# Last lesson recap

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

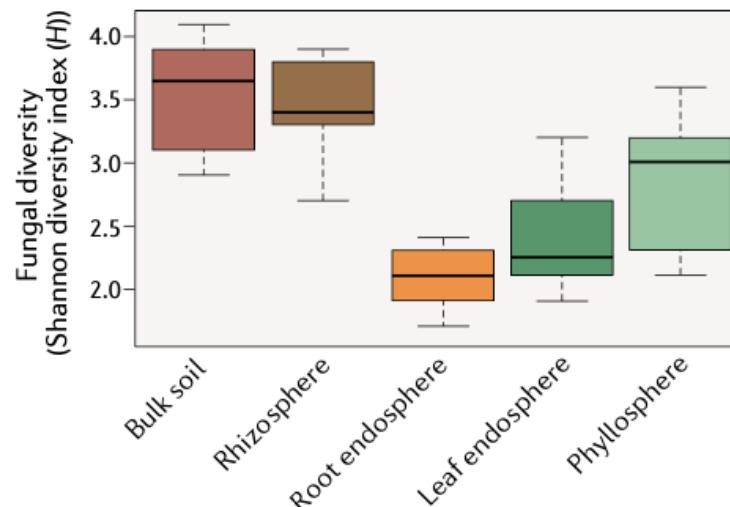
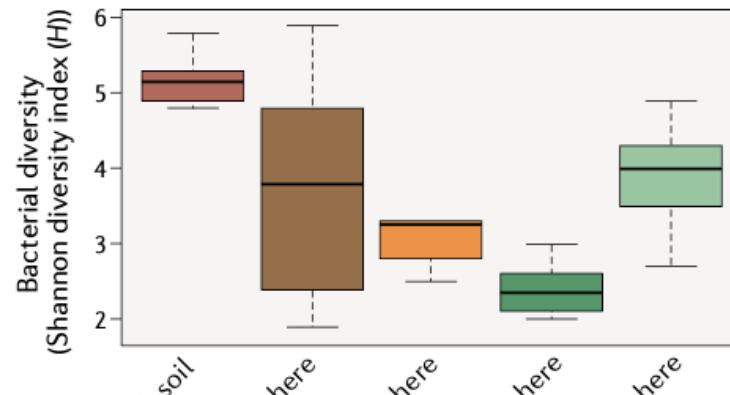
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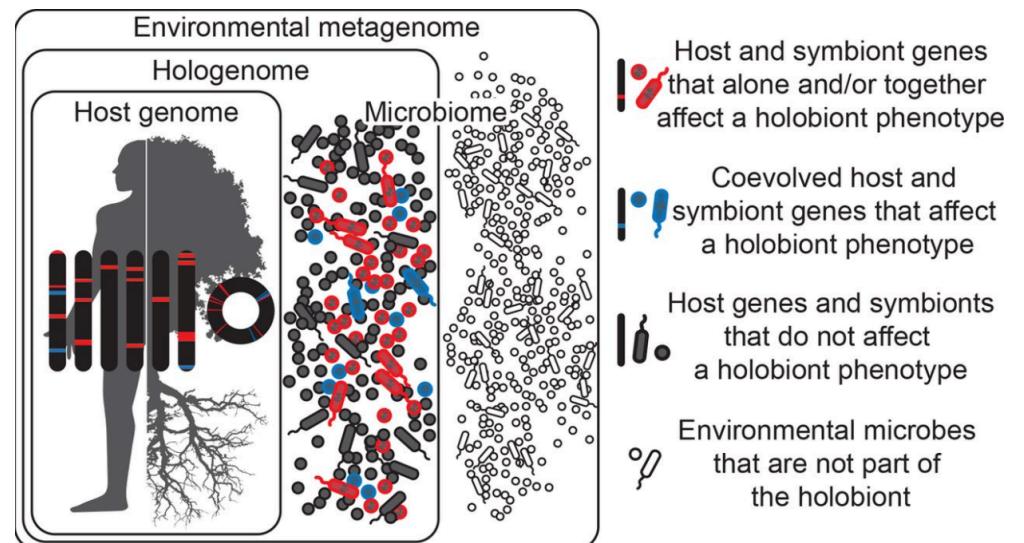
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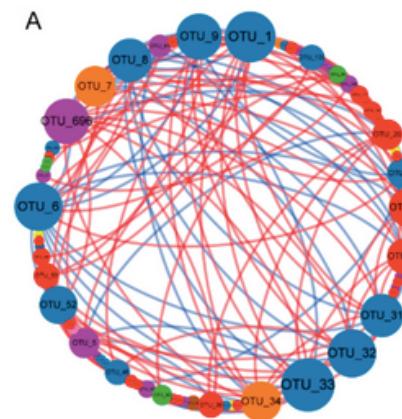
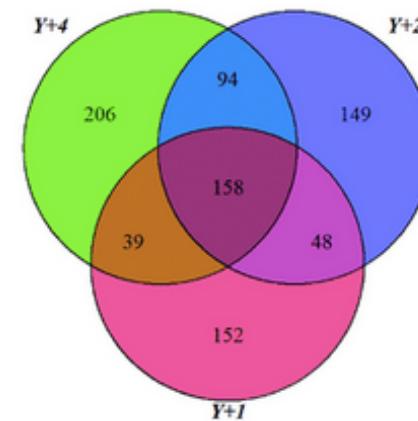
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# Last lesson recap

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# Agenda

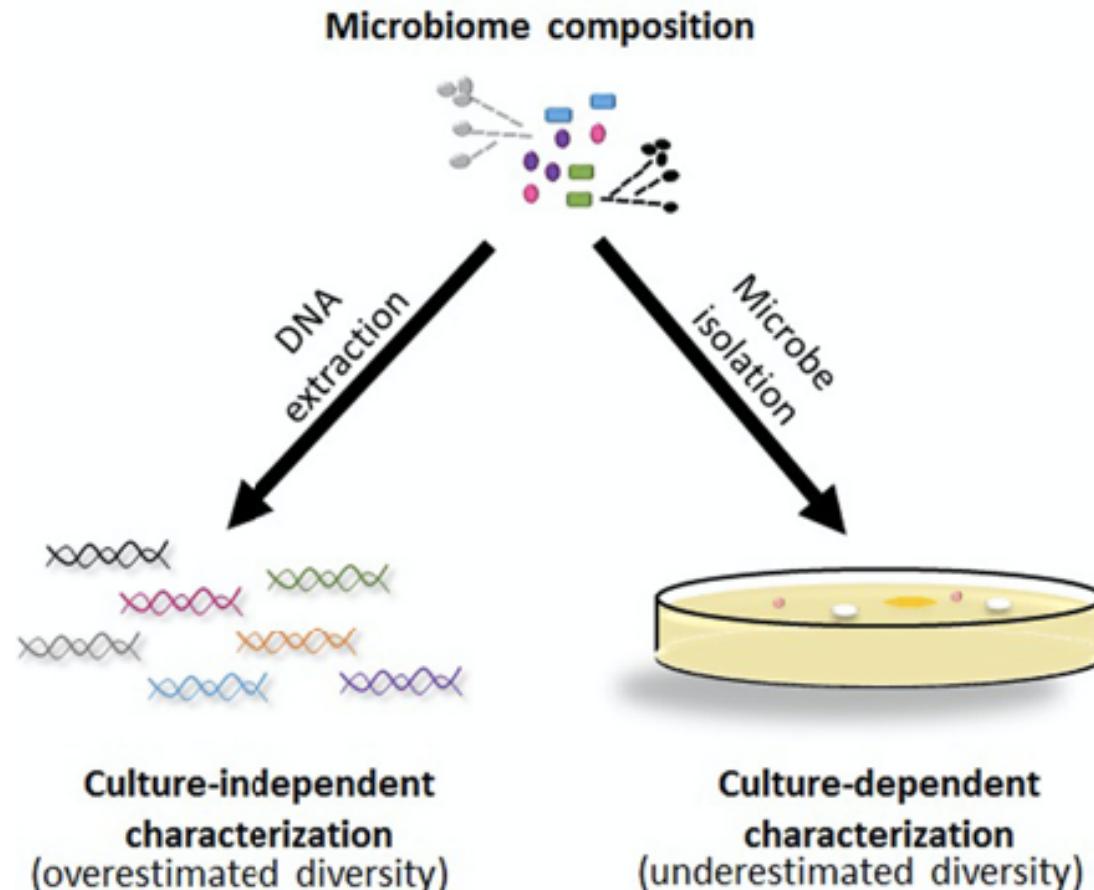
- How do you study the microbiota composition?
- What can the microbiota potentially do?
- What are they doing?

How do you study the microbiota composition?

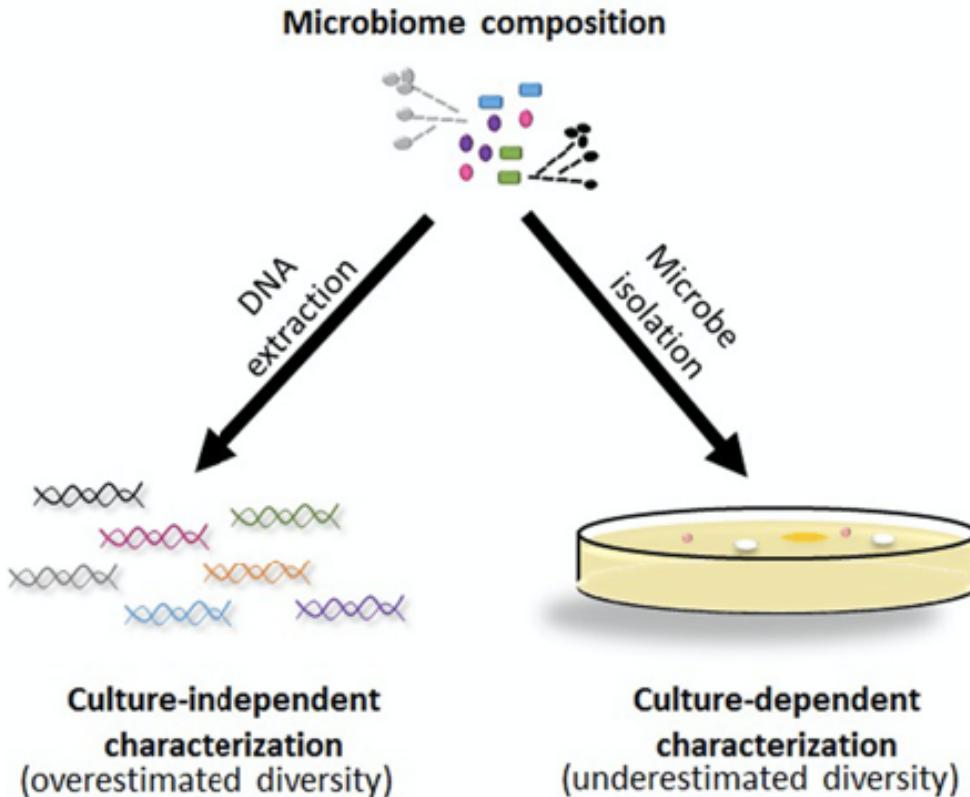
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# How do you study the microbiota composition?

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



# How do you study the microbiota composition?



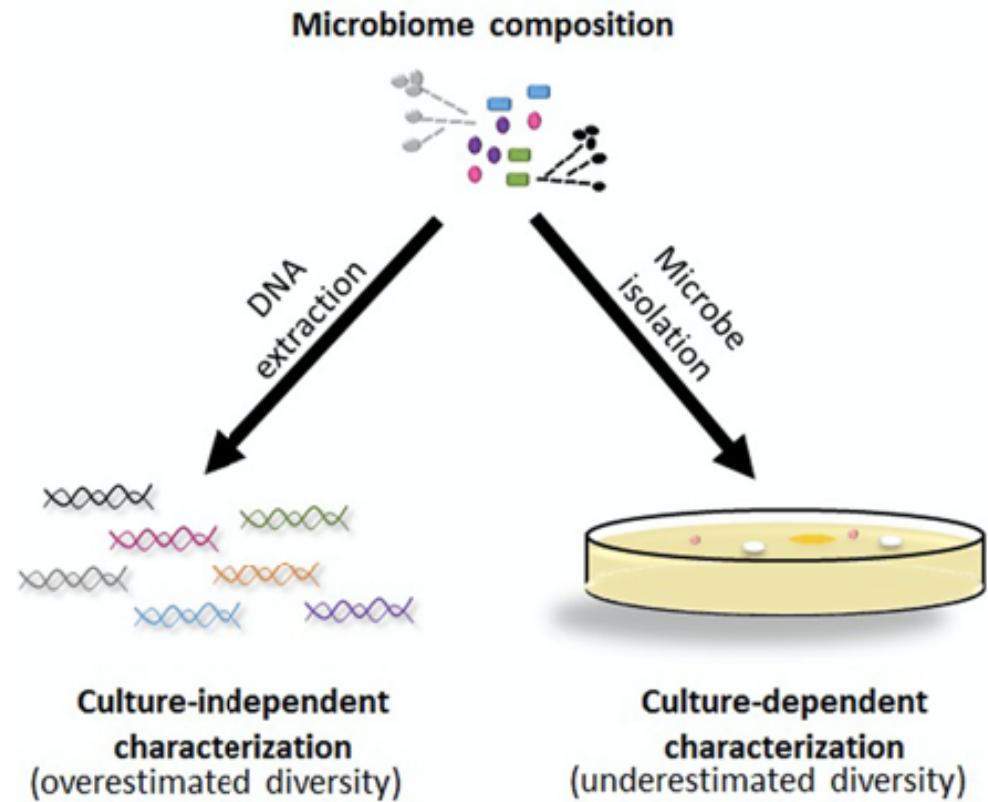
## Culture dependent

- + 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



# 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

What is it the  
**DNA barcoding?**

# How do you study the microbiota composition?

## Microbial DNA metabarcoding

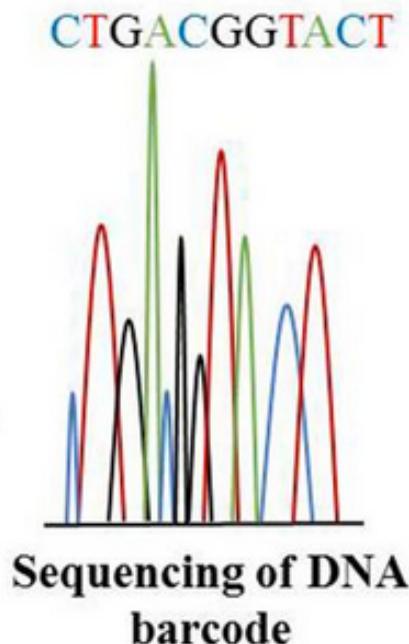
- **DNA barcoding** is a method of species identification using a short section of DNA from a specific gene
- An individual sequence can be used to uniquely identify an organism by comparison with a reference library of classified DNA sequences

# 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



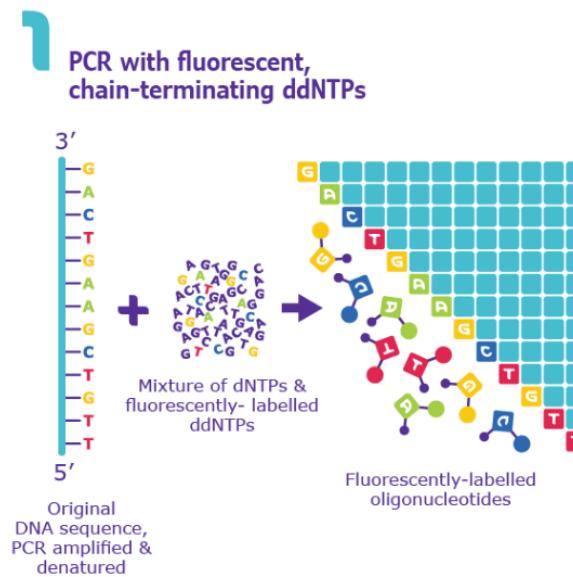
(Mosa et al., 2019)

# How do you study the microbiota composition?

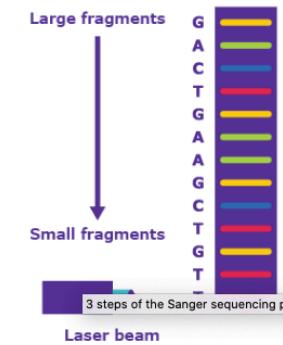
## Sequencing

### Sanger Sequencing

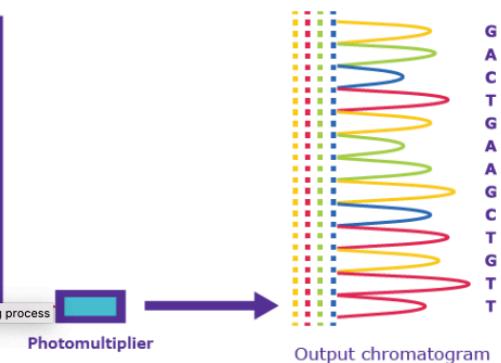
Sanger sequencing, also known as the "chain termination method", is a method for determining the nucleotide sequence of DNA. The method was developed by two time Nobel Laureate Frederick Sanger and his colleagues in 1977, hence the name the Sanger Sequence



2 Size separation by capillary gel electrophoresis



3 Laser excitation & detection by sequencing machine



# How do you study the microbiota composition?

## Microbial DNA metabarcoding

### Marker selection

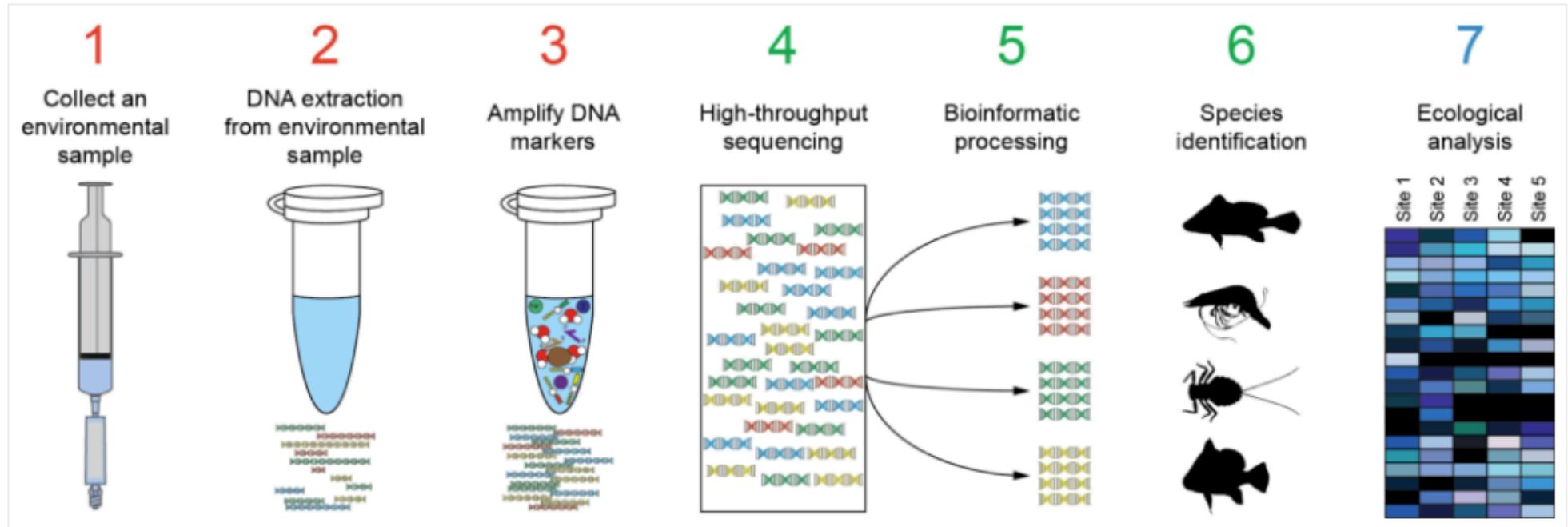
Organism	Region
animals	cytochrome c oxidase I
fungi	internal transcribed spacer (ITS) rRNA
plants	RuBisCO
prokaryotes	16S rRNA
microbial eukaryotes	18S rRNA

When barcoding is used to identify organisms from a sample containing DNA

# How do you study the microbiota composition?

## Microbial DNA metabarcoding

### Metabarcoding process



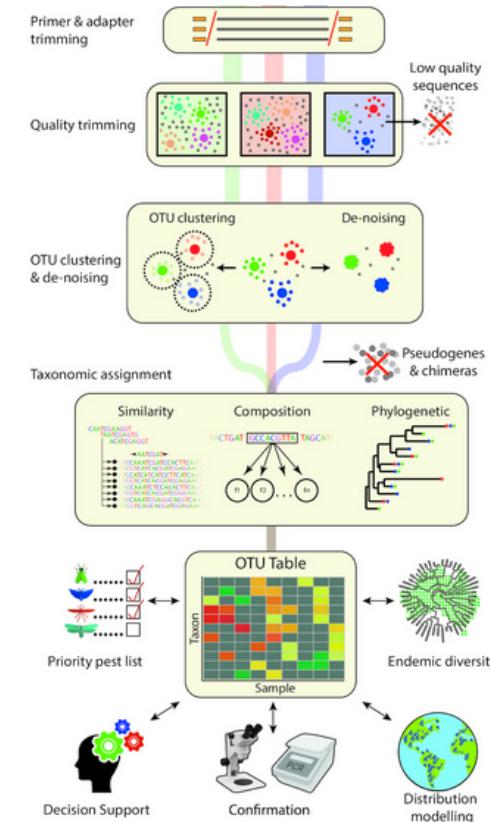
Metabarcoding workflow. Source: <http://www.naturemetrics.co.uk>

# How do you study the microbiota composition?

## Microbial DNA metabarcoding

### Bioinformatic analysis

- 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**)



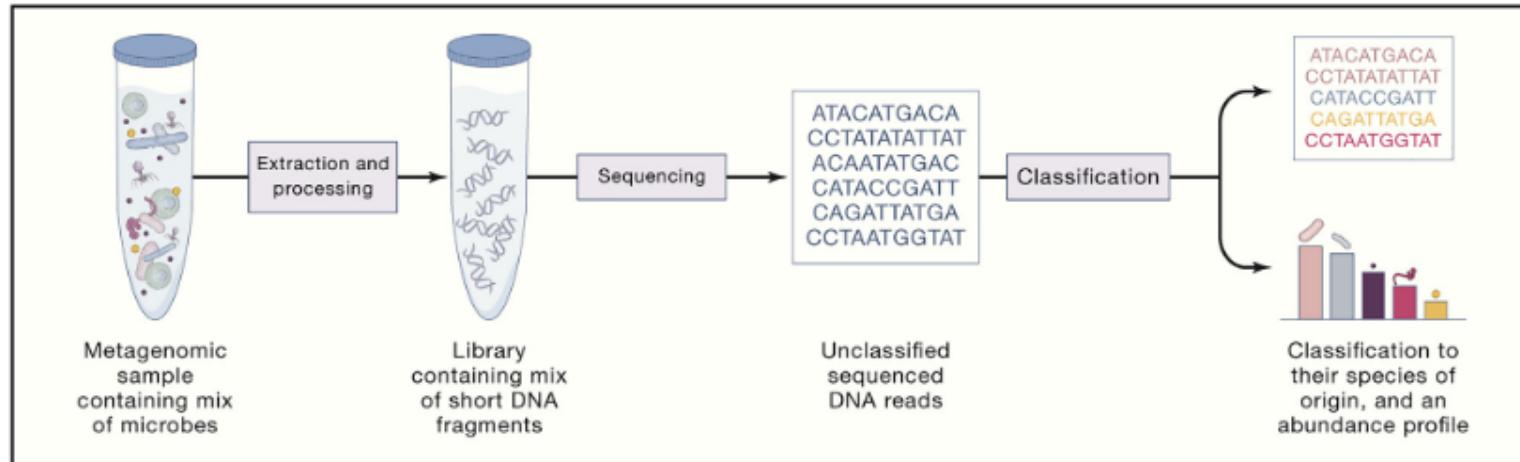
(Jesse et al., 2018)

# What can the microbiota potentially do?

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# What can the microbiota potentially do?

## Metagenomics

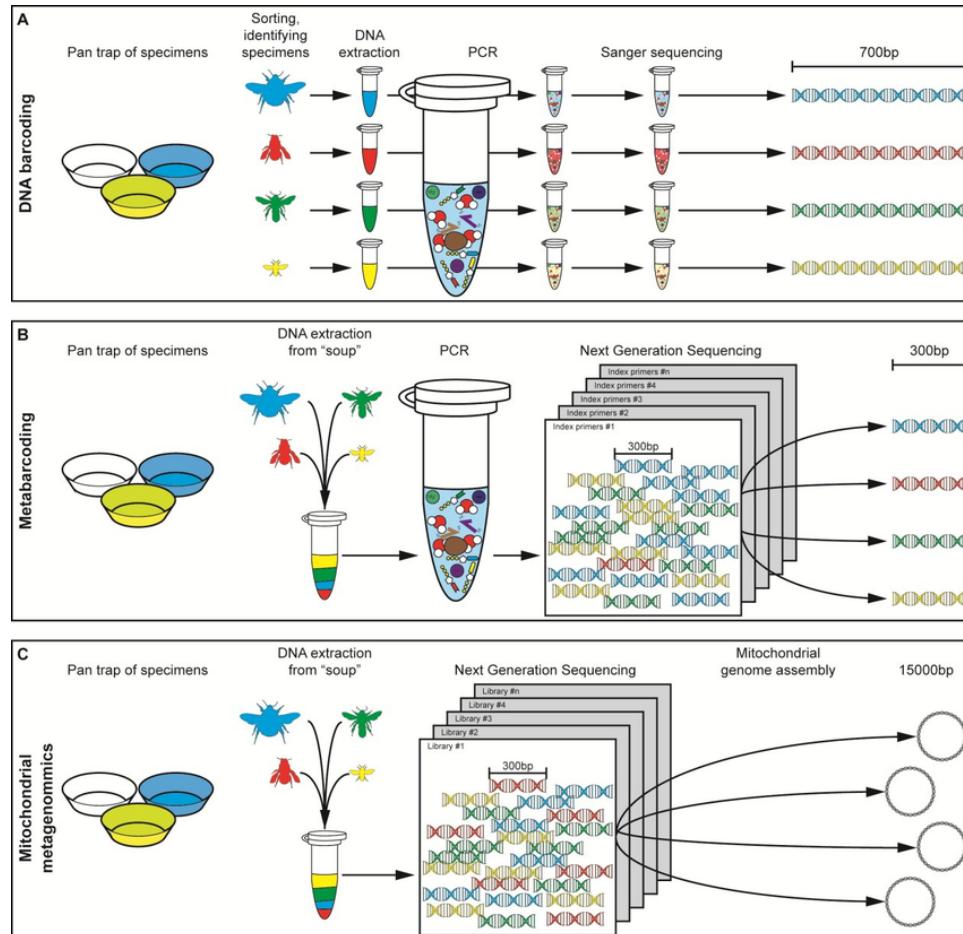


(Ye, Siddle, Park, and Sabeti, 2019)

- **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**

# 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

(Gill, Baldock, Brown, Cresswell, Dicks, Fountain, Garratt, Gough,

# What can the microbiota potentially do?

# What can the microbiota potentially do?

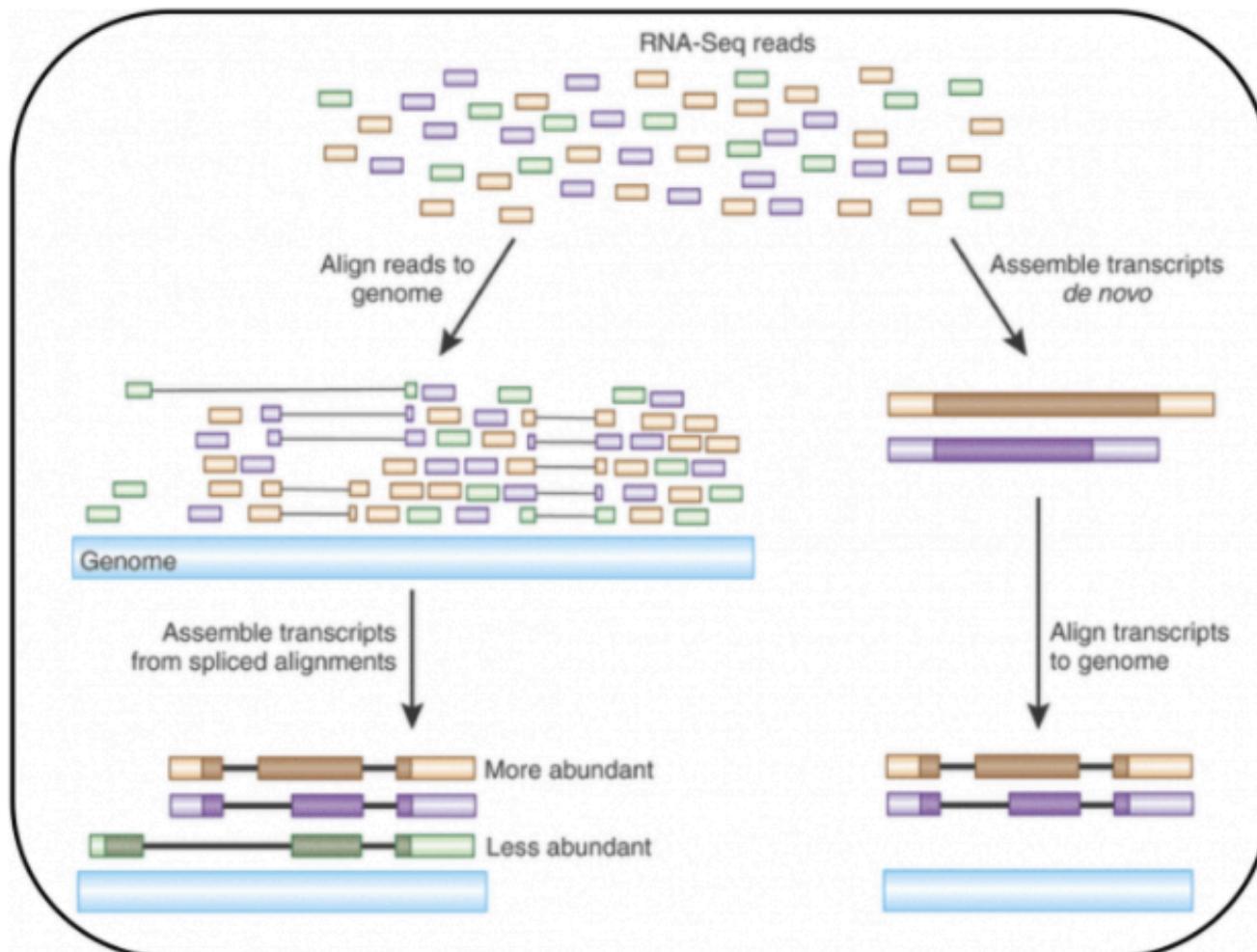
Illumina Sequencing by Synthesis



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

# What can the microbiota potentially do?

## Genomes assembly

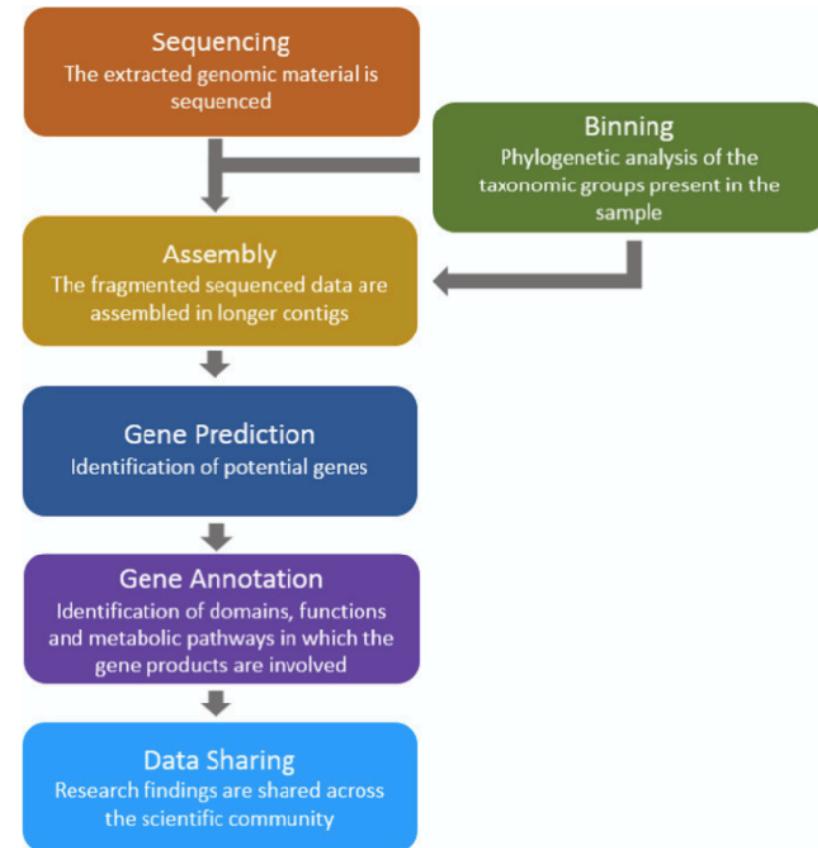


# What can the microbiota potentially do?

## Functional Metagenomics Analysis

Workflow:

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

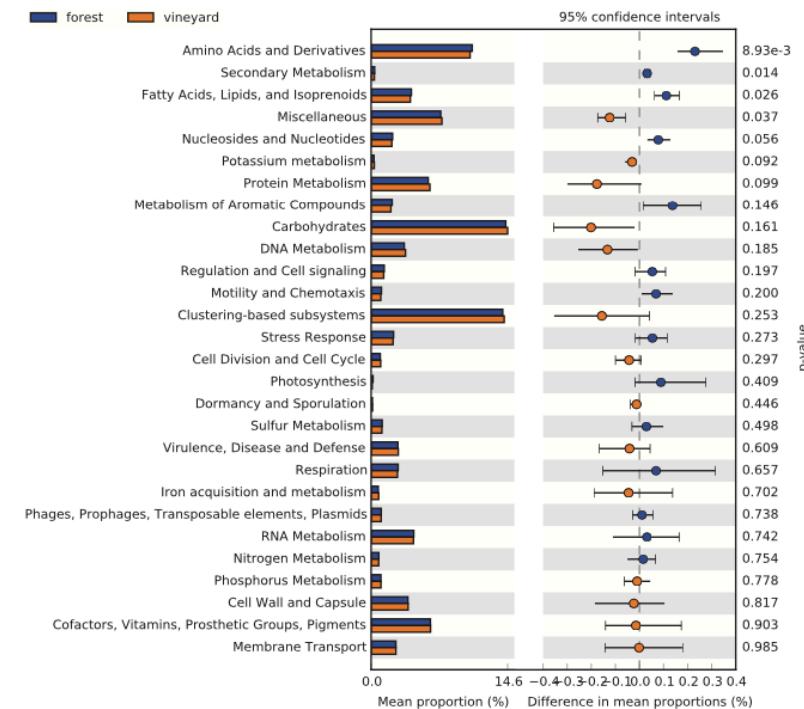


# What can the microbiota potentially do?

## Functional Metagenomics Analysis

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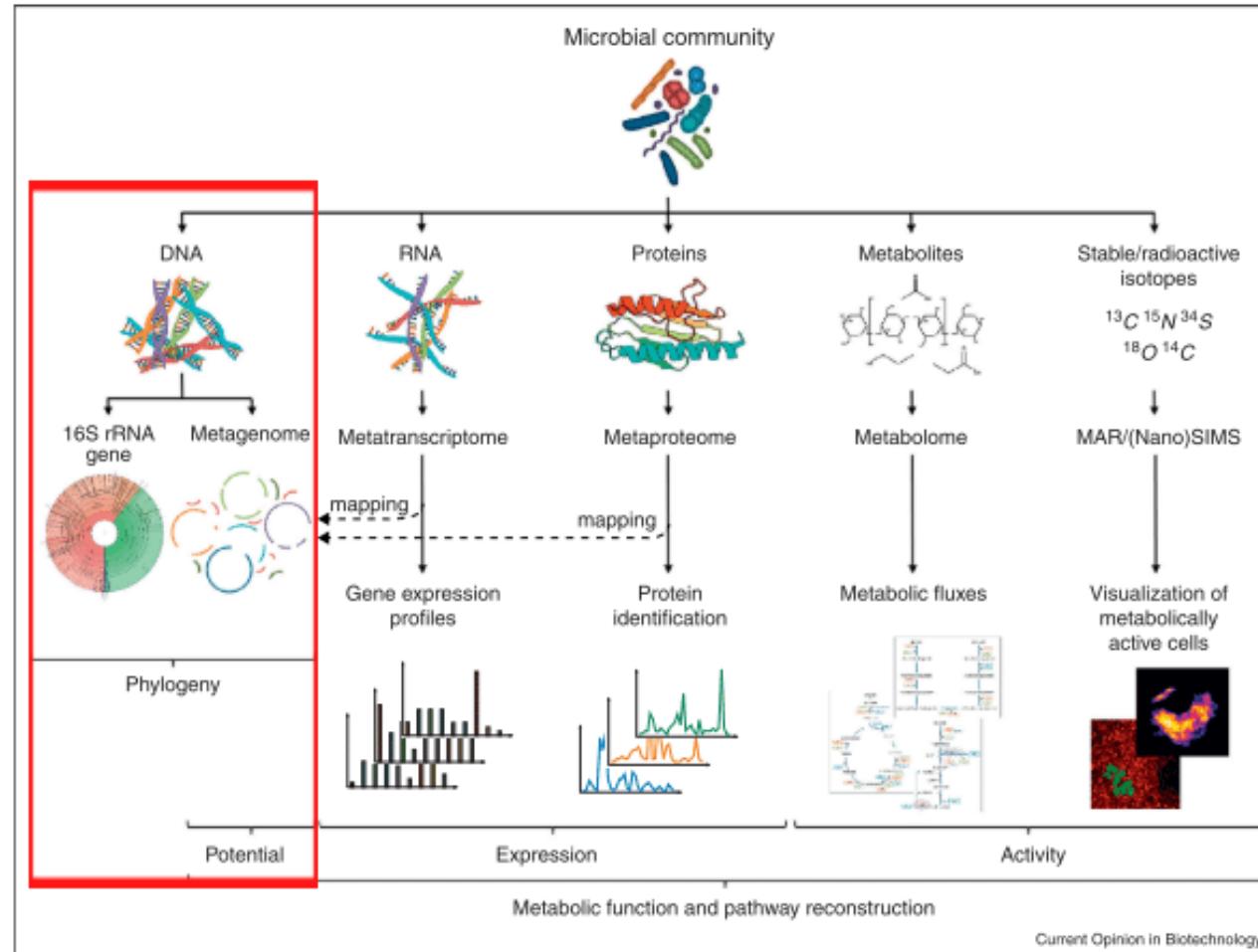
**Figure 4** Functional categories found in soil microbial communities. Bar plot showing the mean proportion (%) of functional categories found in soil microbial communities based on the subsystem database. Points indicate the differences between forest and vineyard soils (blue and orange bars, respectively),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

(Castañeda and Barbosa, 2017)

What are they doing?

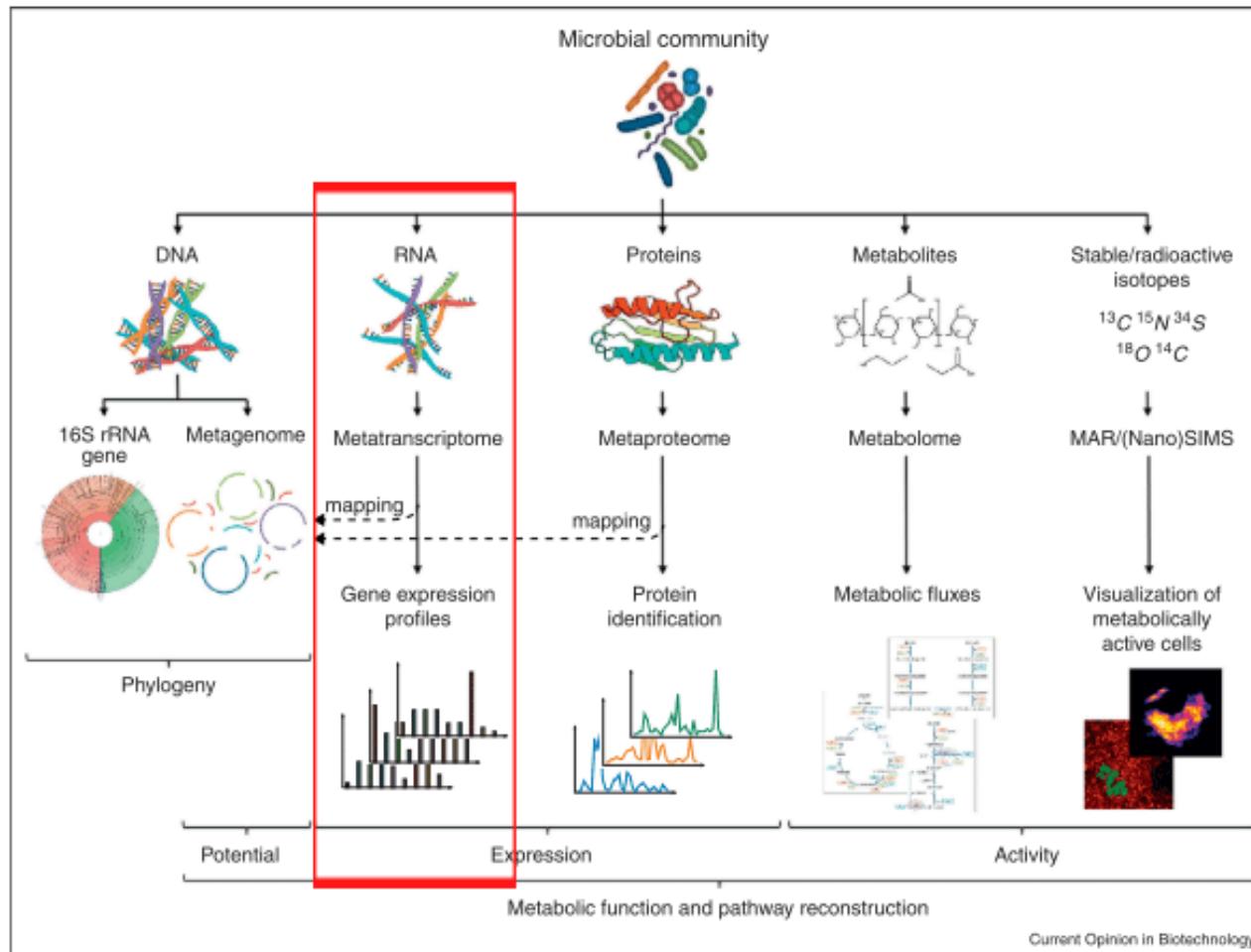
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# What are they doing?



# What are they doing?

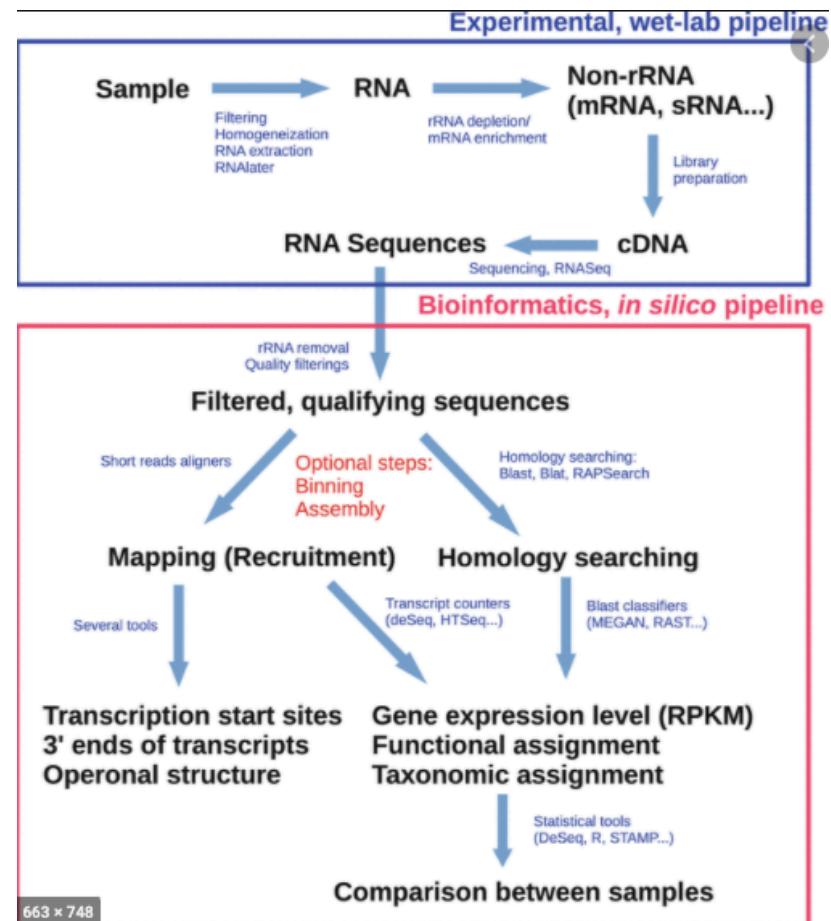
## Metatranscriptomics



# What are they doing?

## Metatranscriptomics

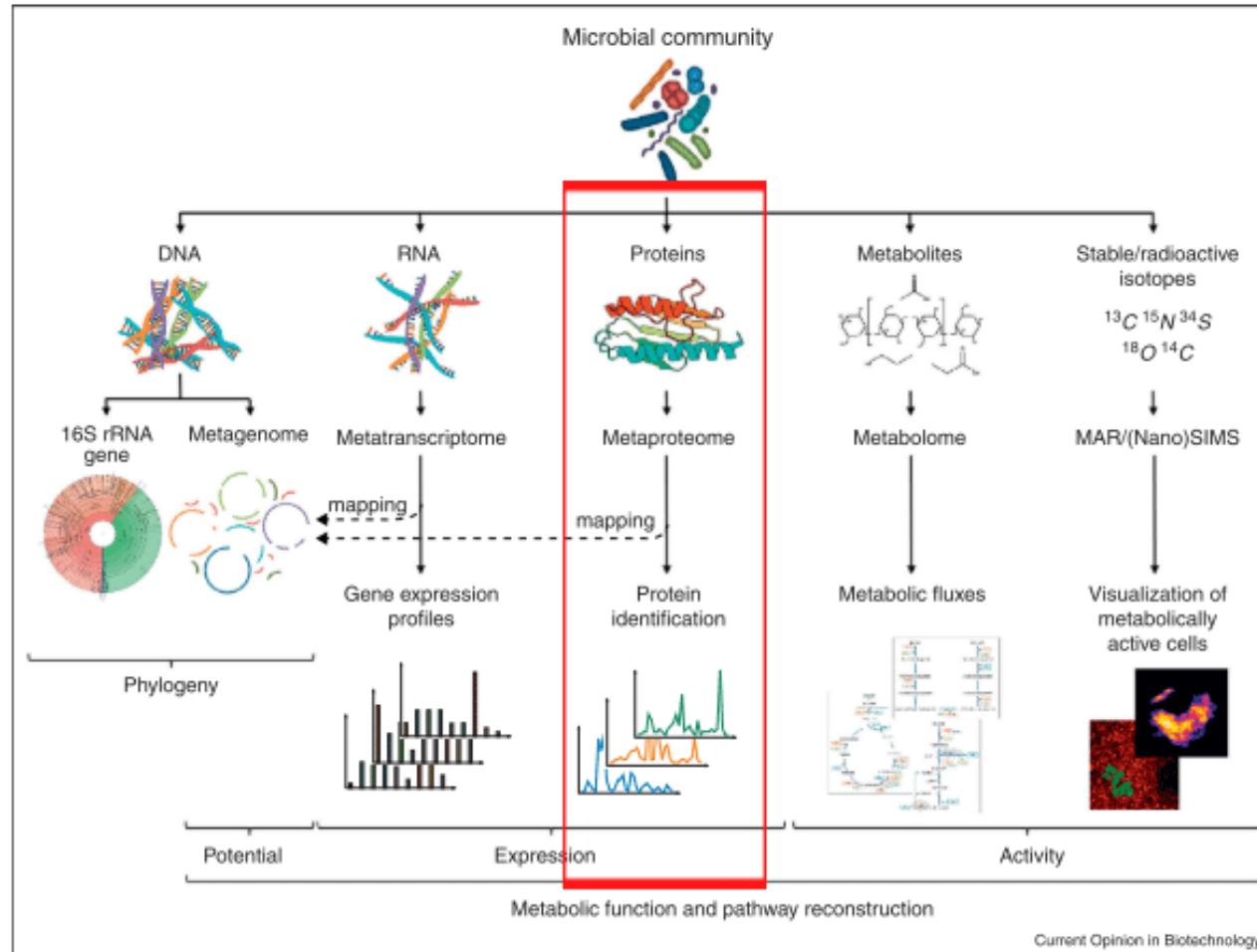
- Metatranscriptomics is the science that studies gene expression of microbes within natural environments
- It allows to obtain whole gene expression profiling of complex microbial communities
- + The advantage of metatranscriptomics, over metagenomics, is that it can provide information about differences 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



(Pérez-Pantoja and Tamames, 2015)

# What are they doing?

## Metaproteomics



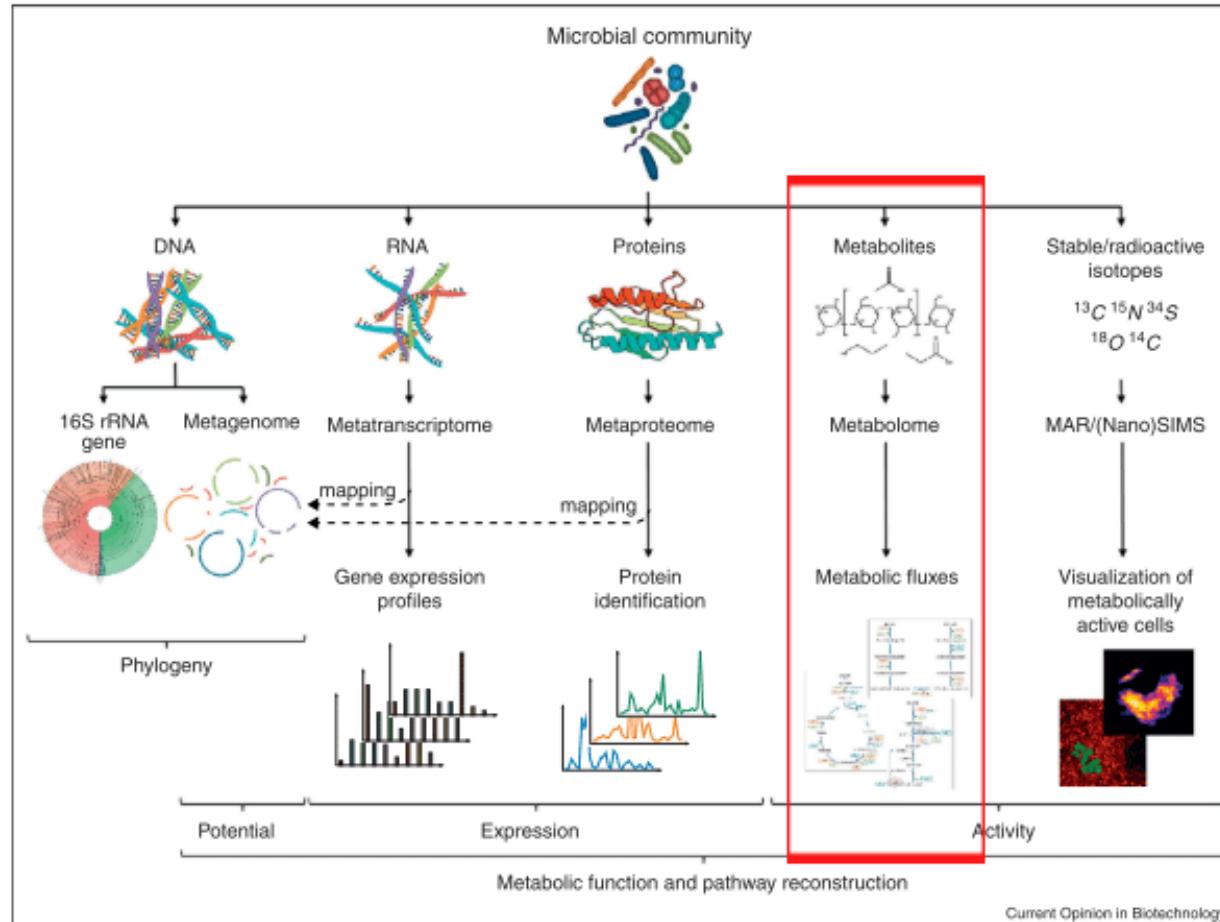
# What are they doing?

## 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

# What are they doing?

## Metabolomics



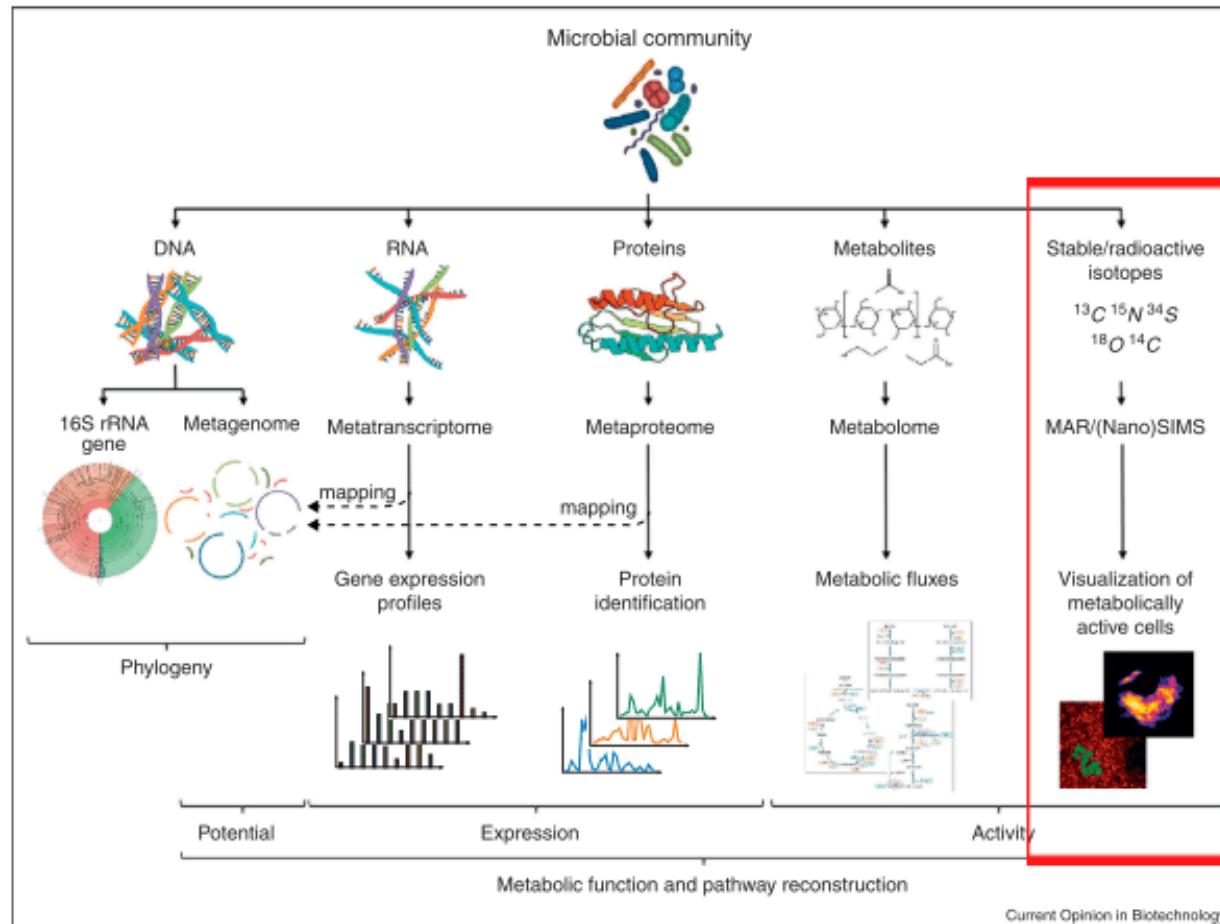
# What are they doing?

## 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.

# What are they doing?

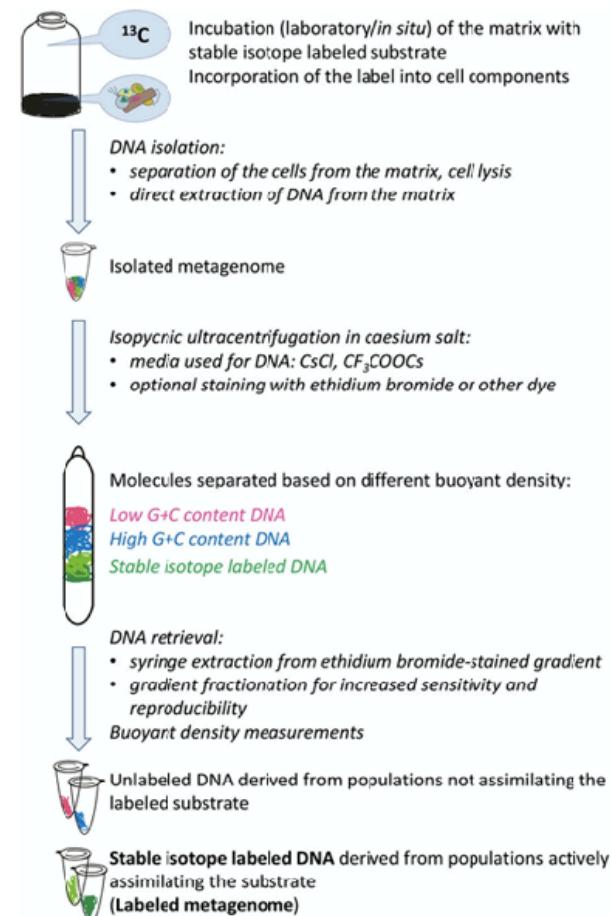
## Stable/radioactive isotopes



# What are they doing?

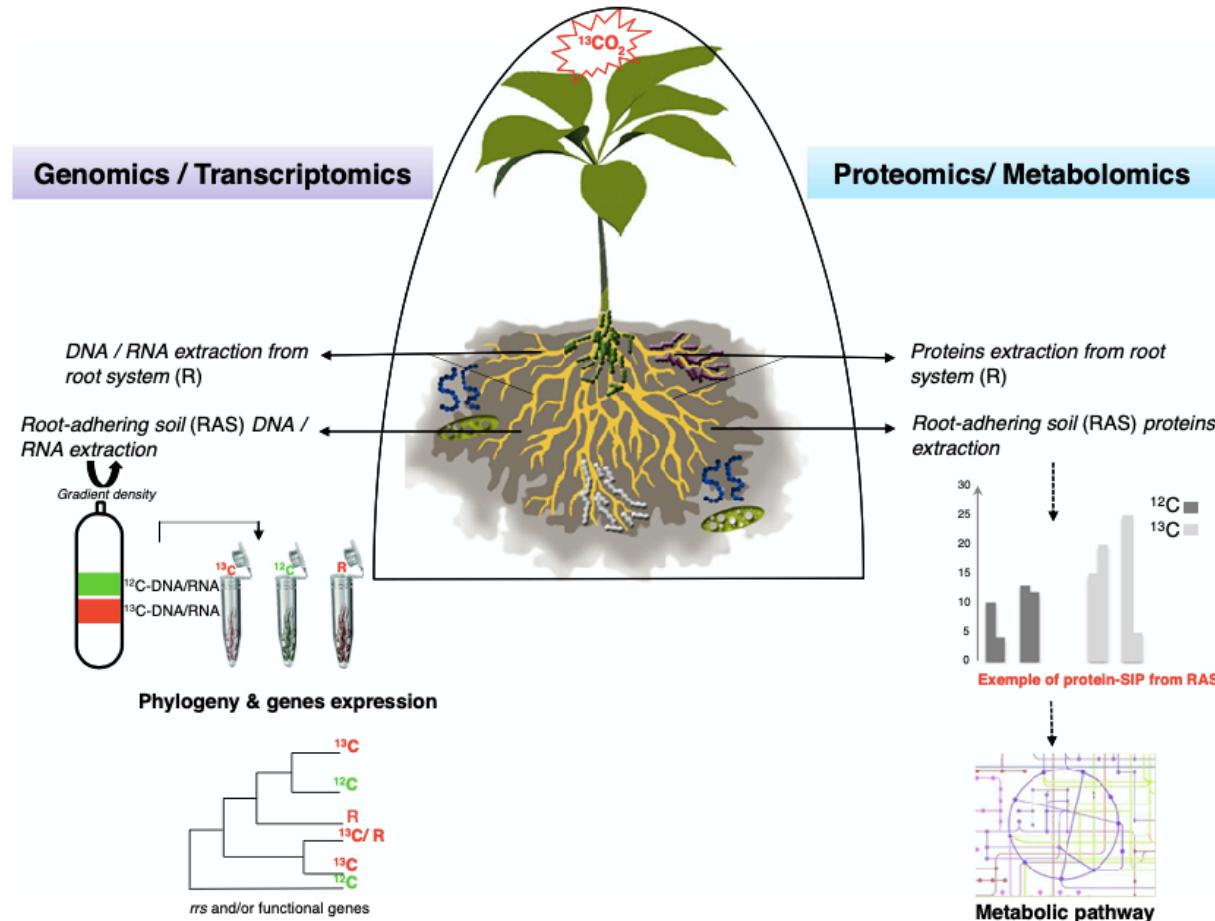
## Stable/radioactive isotopes

- 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

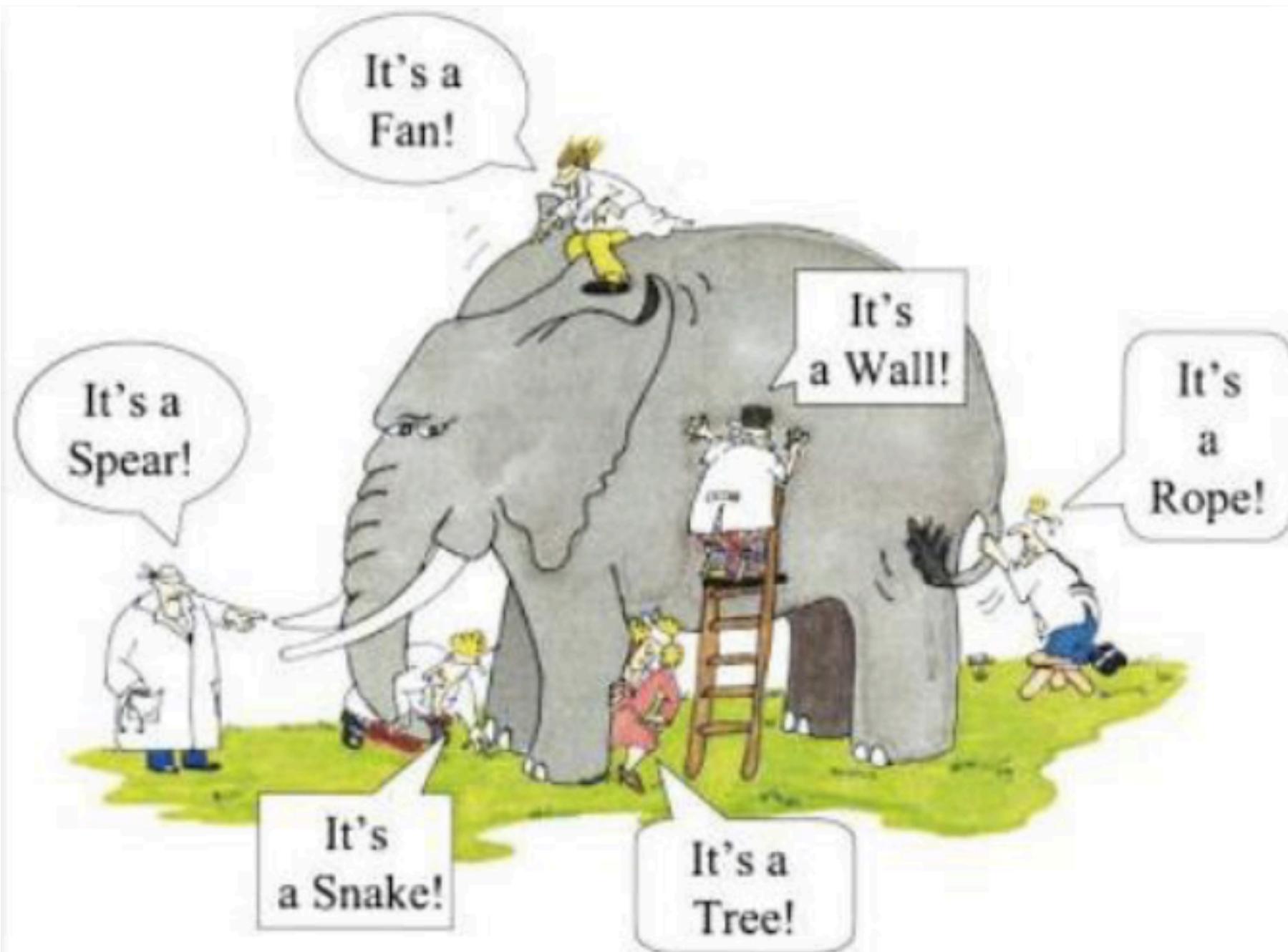


# What are they doing?

Stable/radioactive isotopes

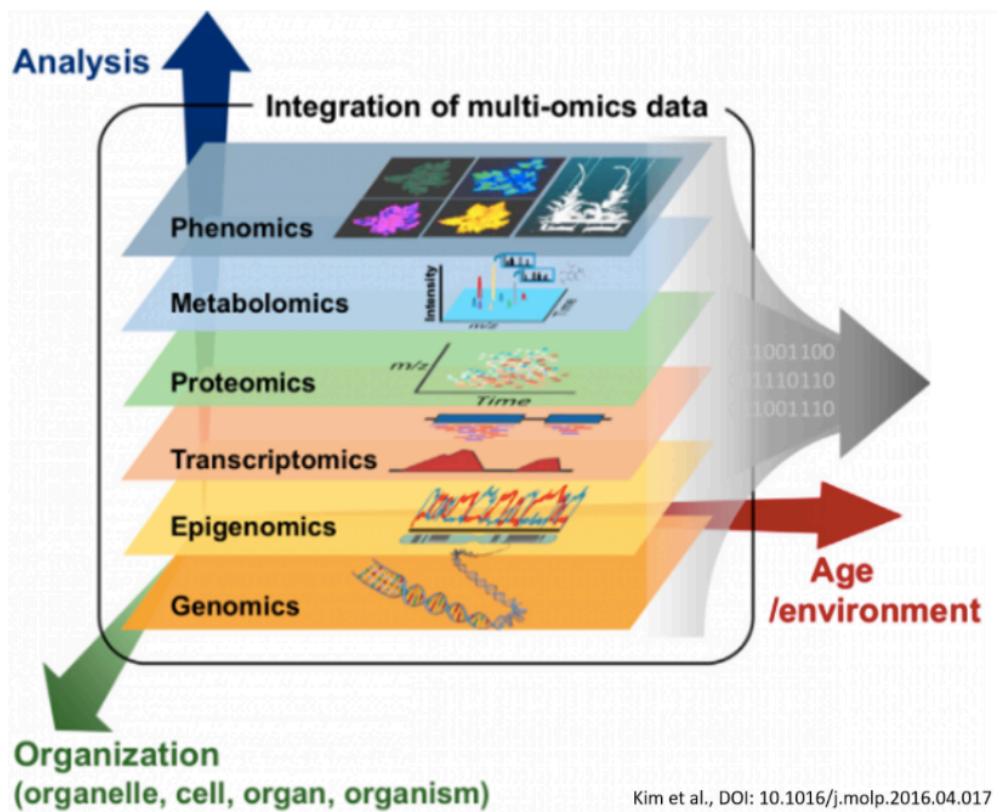


(el Zahar Haichar, Heulin, Guyonnet, et al., 2016)



# 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



Kim et al., DOI: 10.1016/j.molp.2016.04.017

# Questions about the lesson



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