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RECYCLES WORKSHOP

Metagenomics and metabarcoding approaches to describe ecological systems and infer their development

5th, 6th & 7th of July 2022

The predictive functional metagenomic approach in the process studies

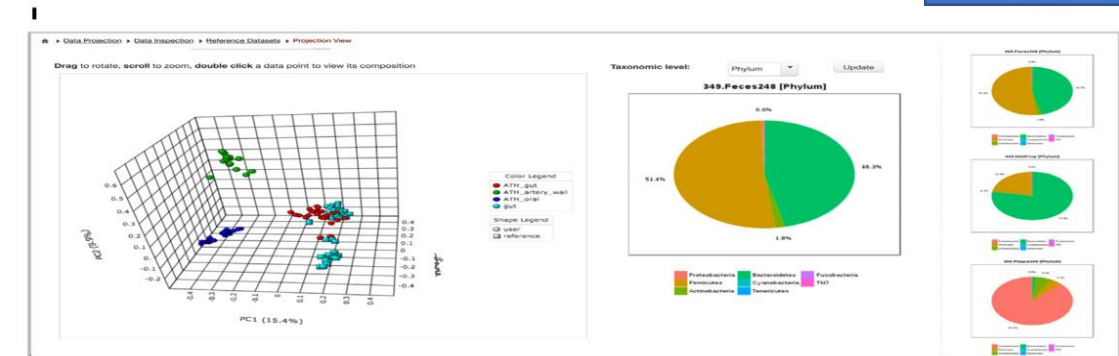
Simone Becarelli



European
Commission



GA: 872053 — H2020 - MSCA - RISE-2019




Feature table (ASV is better)

Phylogenetic tree

Taxonomy table

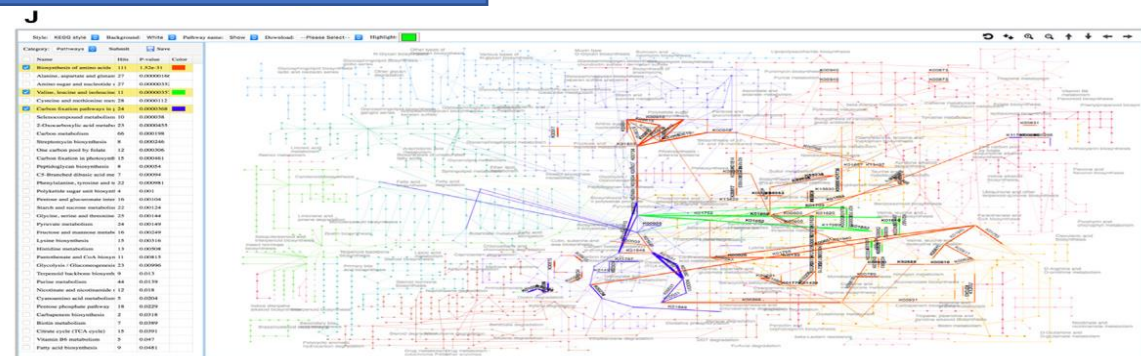
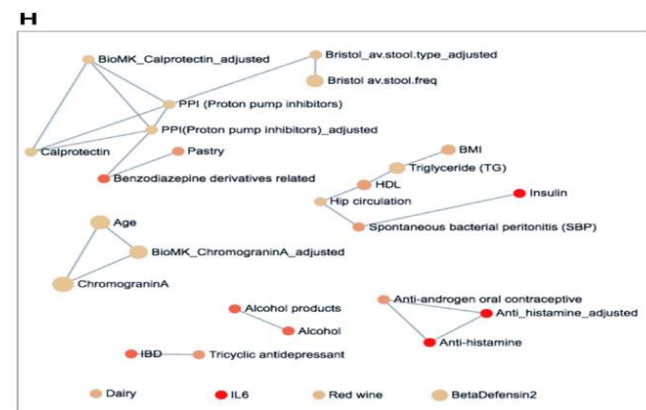
Functional inference

A yellow starburst graphic with a black outline, containing the word "NEW" in bold black capital letters.

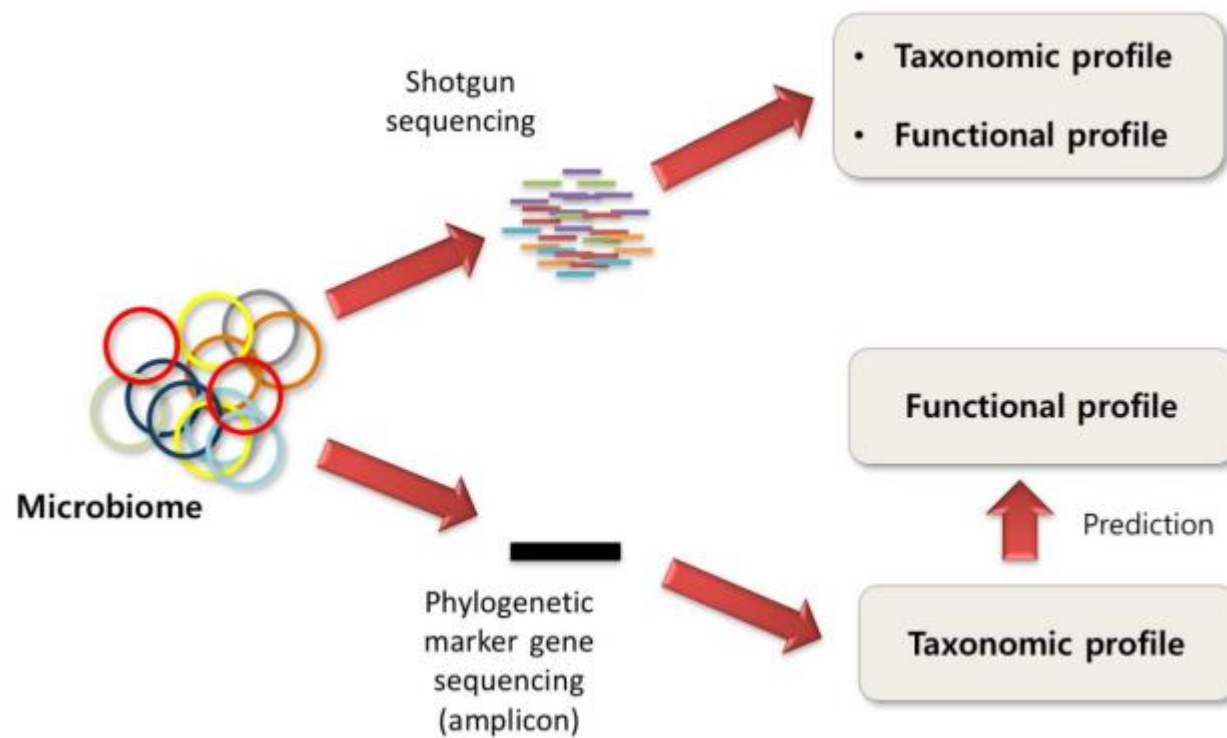
Taxonomy table

Functional inference

NEW



Microbiome sequencing



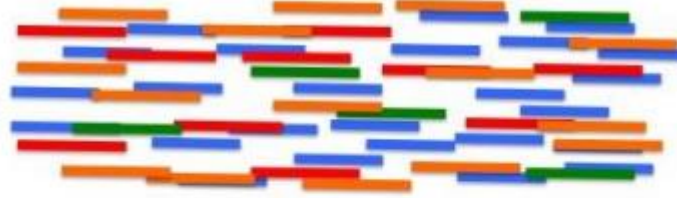
Shotgun sequencing



Bacterial genomes
present in a sample



Genomes cut into small
fragments



Sequencing of many random
fragments from pool of
fragments



DNA
sequences
Computer-
assembled
consensus
sequence



Alignment of DNA sequences with a computer
program to create a larger consensus sequence

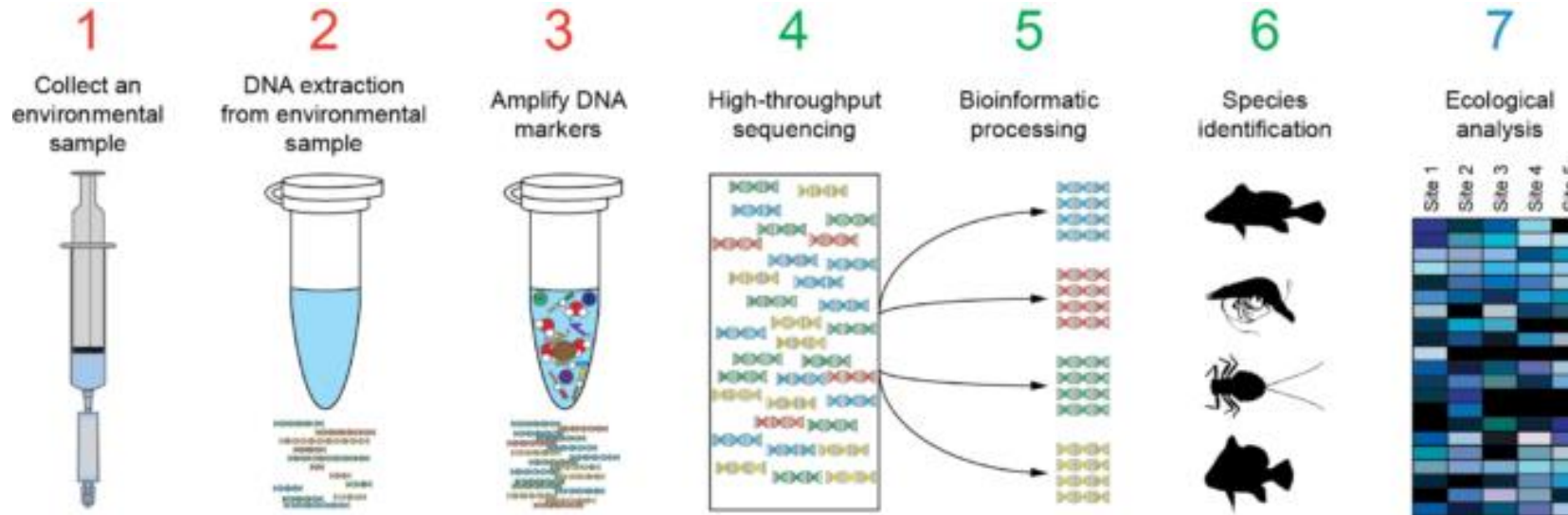
Shotgun Pros

- No primer bias
- Direct functional profile
- MAG reconstruction

Shotgun Cons

- High DNA amount and sequencing effort
- Loses rare sequences
- High computational demand
- Can be severely contaminated

Metabarcoding sequencing



Metabarcoding Pros

- Higher sequencing depth (multiplexing)
- Standard pipelines
- Low DNA amount (PCR dependent)

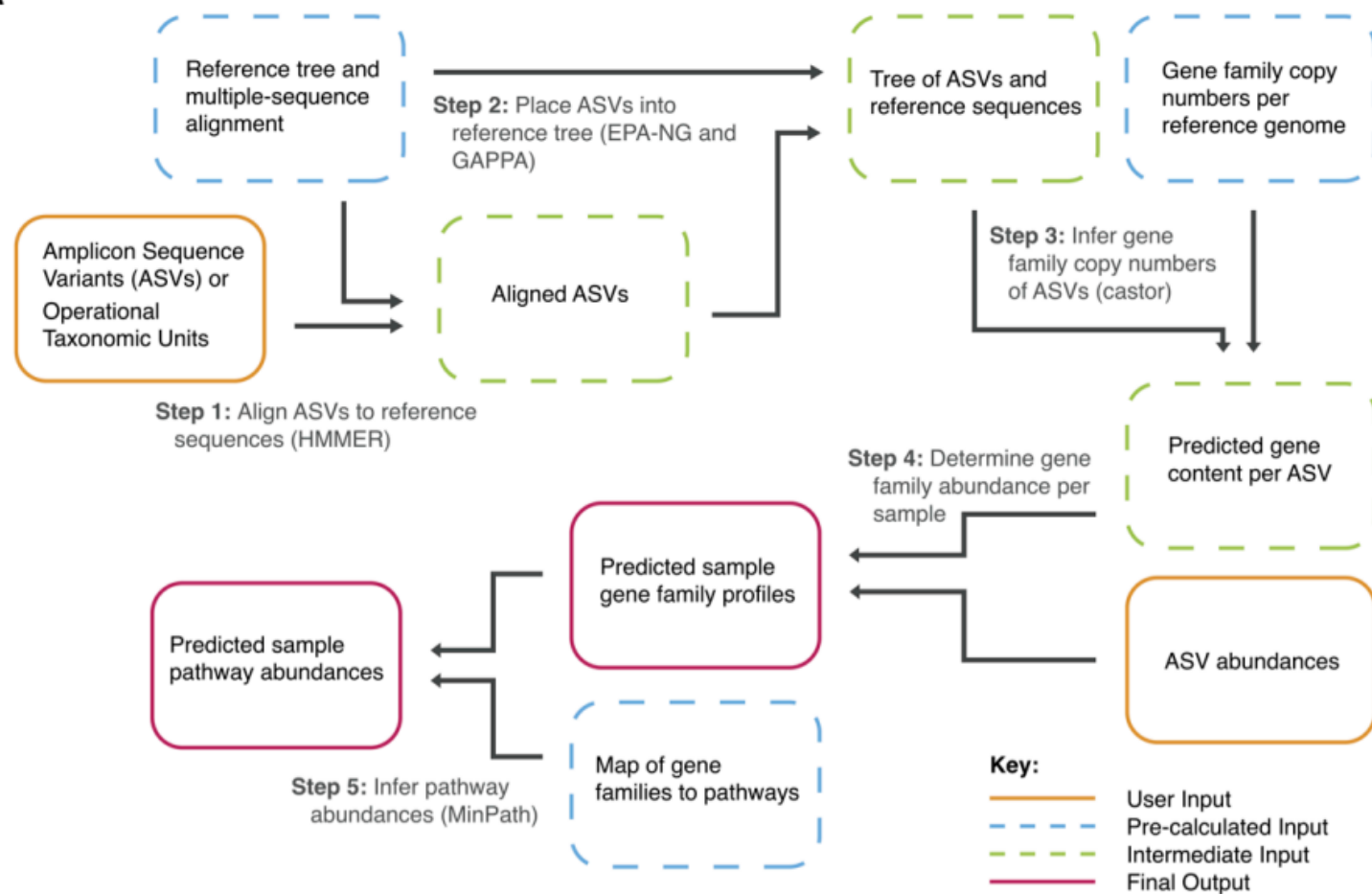
Metabarconding Cons

- Primer bias
- PCR can perturbate real composition
- No direct functional profile

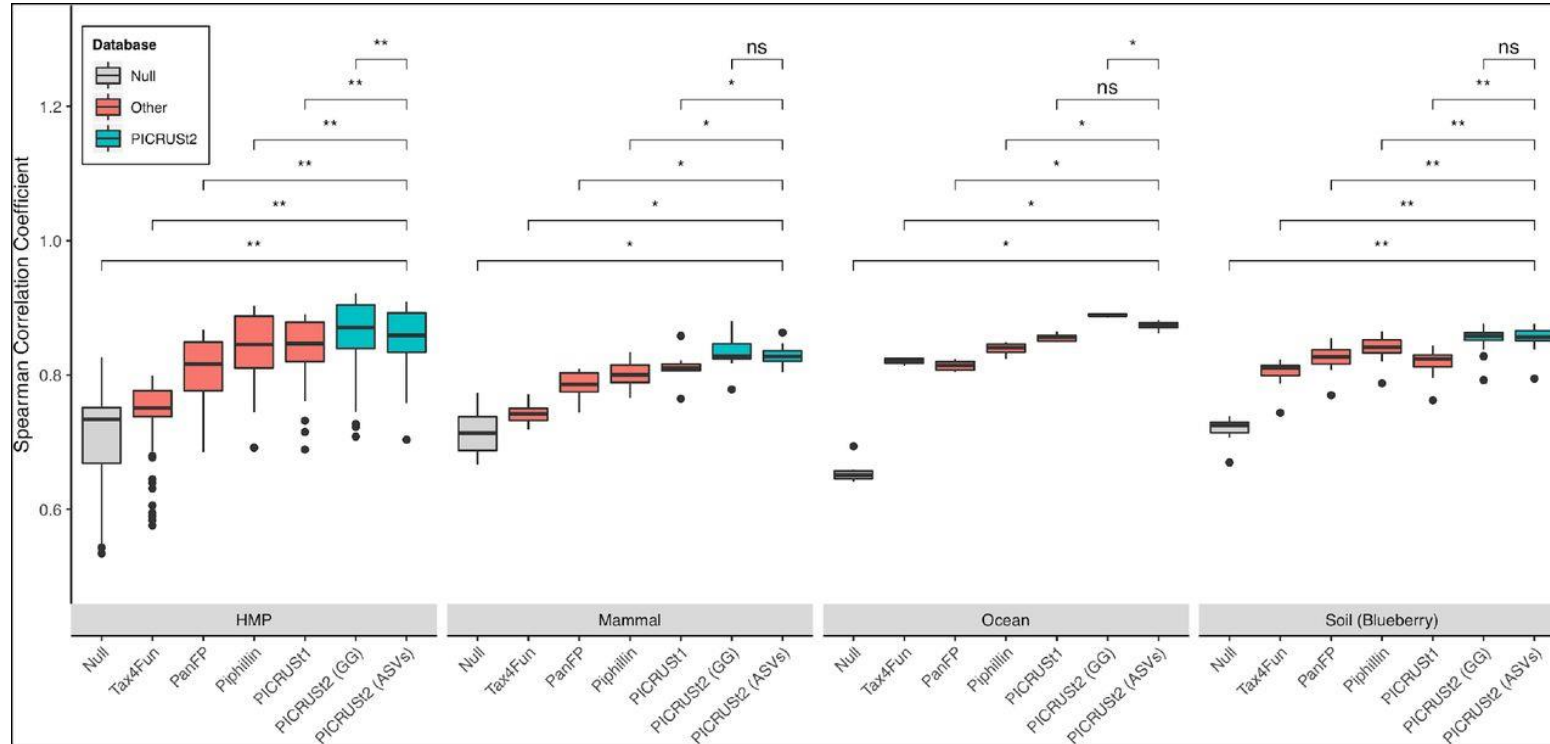
Functional inference: PICRUSt2



A



PICRUSt2 performances v.s. Shotgun prediction



results of PICRUSt2 e against gold-standard shotgun metagenomic sequencing (MGS). human microbiome project (HMP, n=116), mammalian stool (n=8), ocean water (n=6), and blueberry soil (n=22)

PICRUSt2: An improved and extensible approach for metagenome inference

Gavin M. Douglas, Vincent J. Maffei, Jesse Zaneveld, Svetlana N. Yurgel, James

R. Brown, Christopher M. Taylor, Curtis Huttenhower, Morgan G. I. Langille

bioRxiv 672295; doi: <https://doi.org/10.1101/672295>

Case study: DF and AD of Organic Fraction of Municipal Solid Waste



Step1



Step2



Step3



Step4



1 l

>>

20 l

>>

600 l

>>

3000 m³



Regione Toscana



FAS
Fondo Aree
Sottoutilizzate
2007-2013

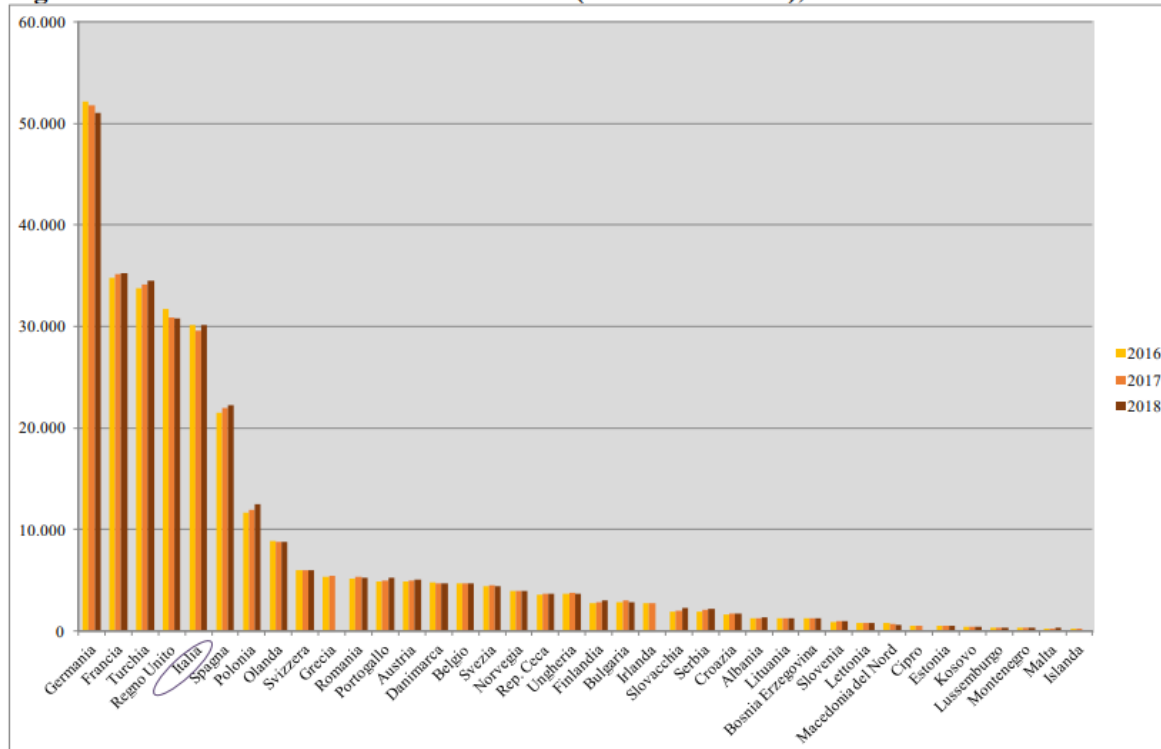


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Municipal Solid Waste management

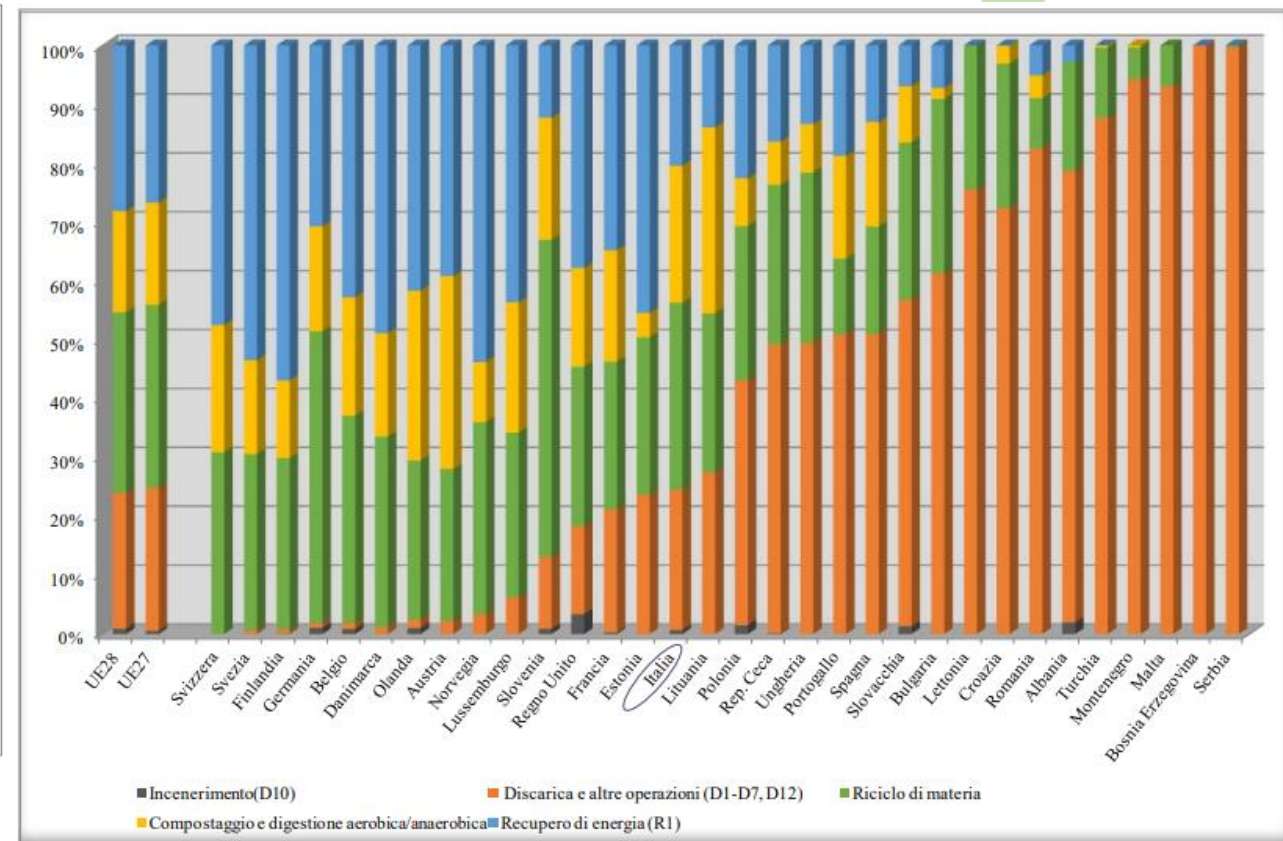


Figura 1.3 – Produzione totale di RU nell'UE (tonnellate*1.000), anni 2016 - 2018



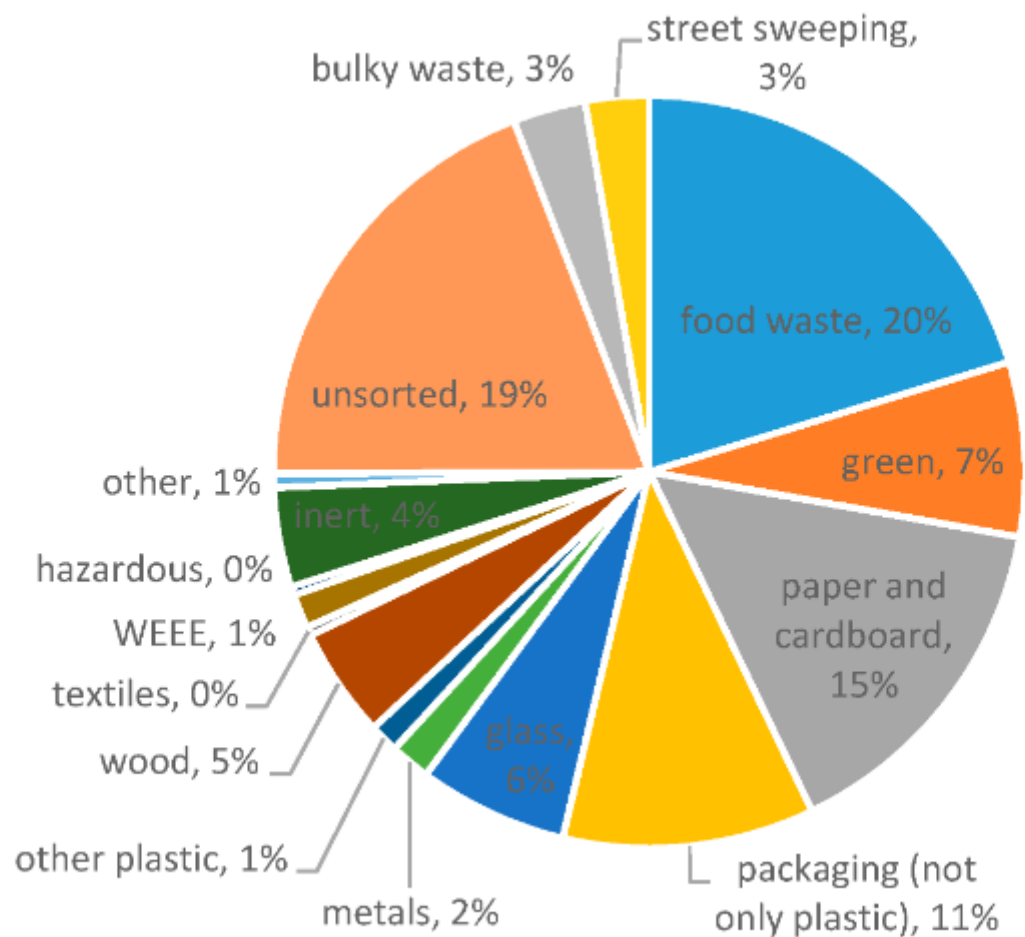
RU = rifiuti urbani

Fonte: elaborazioni ISPRA su dati Eurostat



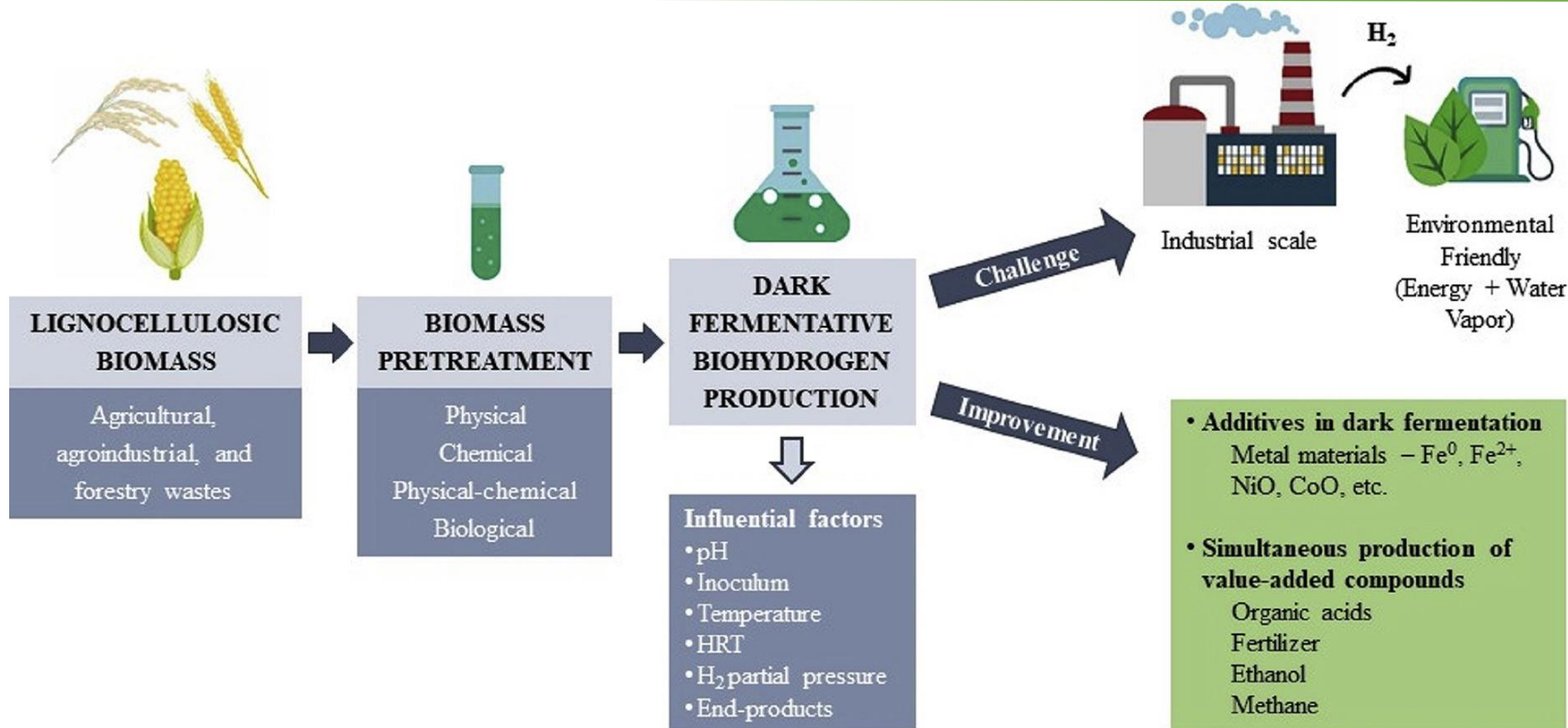
Fonte: elaborazioni ISPRA su dati Eurostat

MSW composition (Trento 2016)



47 % is organic and
can be fermented

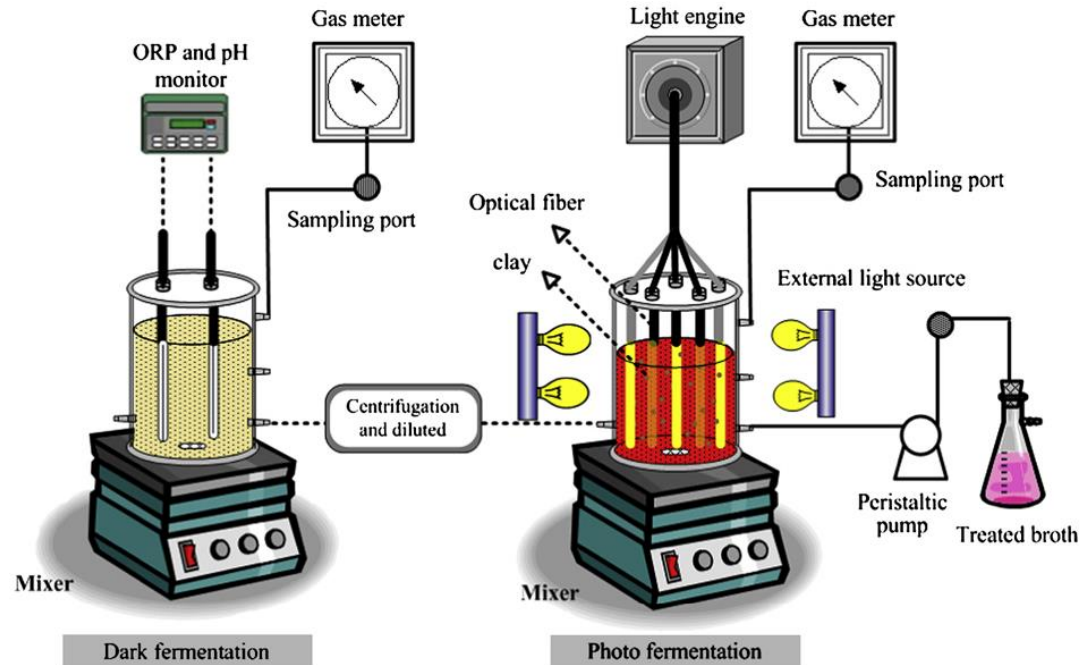
Dark Fermentation



Dark fermentation alone

- Poor H_2 production
- High SCVFA production
- Easy to start-up, hard to be optimised

Dark Fermentation enhancement- Light fermentation



Photofermentation (light fermentation)

VFA converted in H_2

Rhodobacter, *Rhodobium*,
Rhodopseudomonas, *Rhodospirillum*

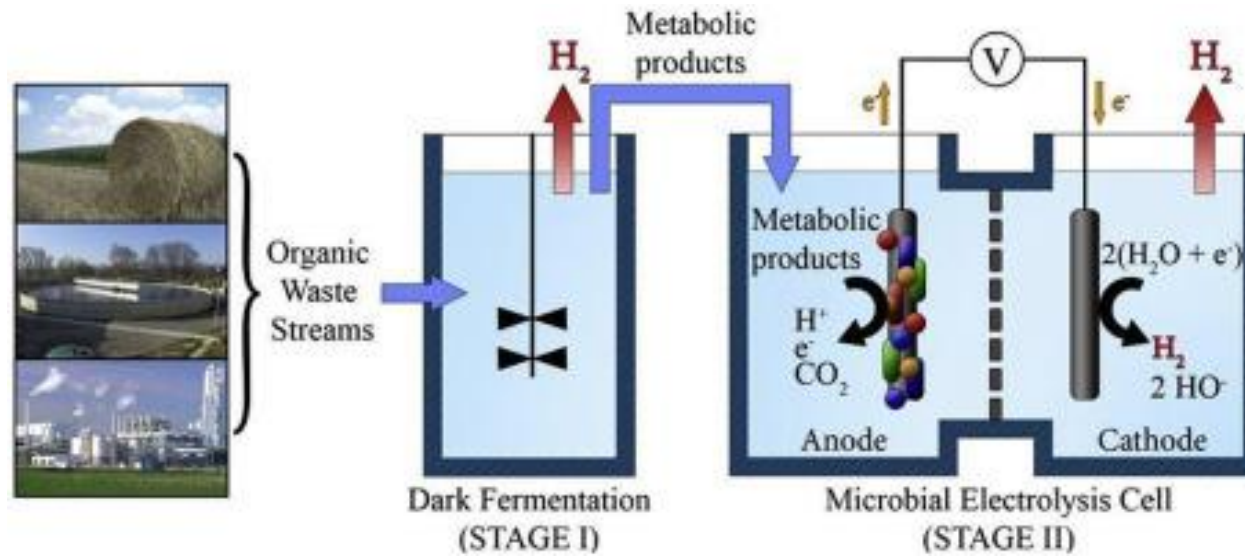
Biophotolysis

CO_2 is converted to hydrogen

Chladimonas reinwardtii

Spatial separation between H_2 and O_2
production needed

Dark Fermentation enhancement- MEC



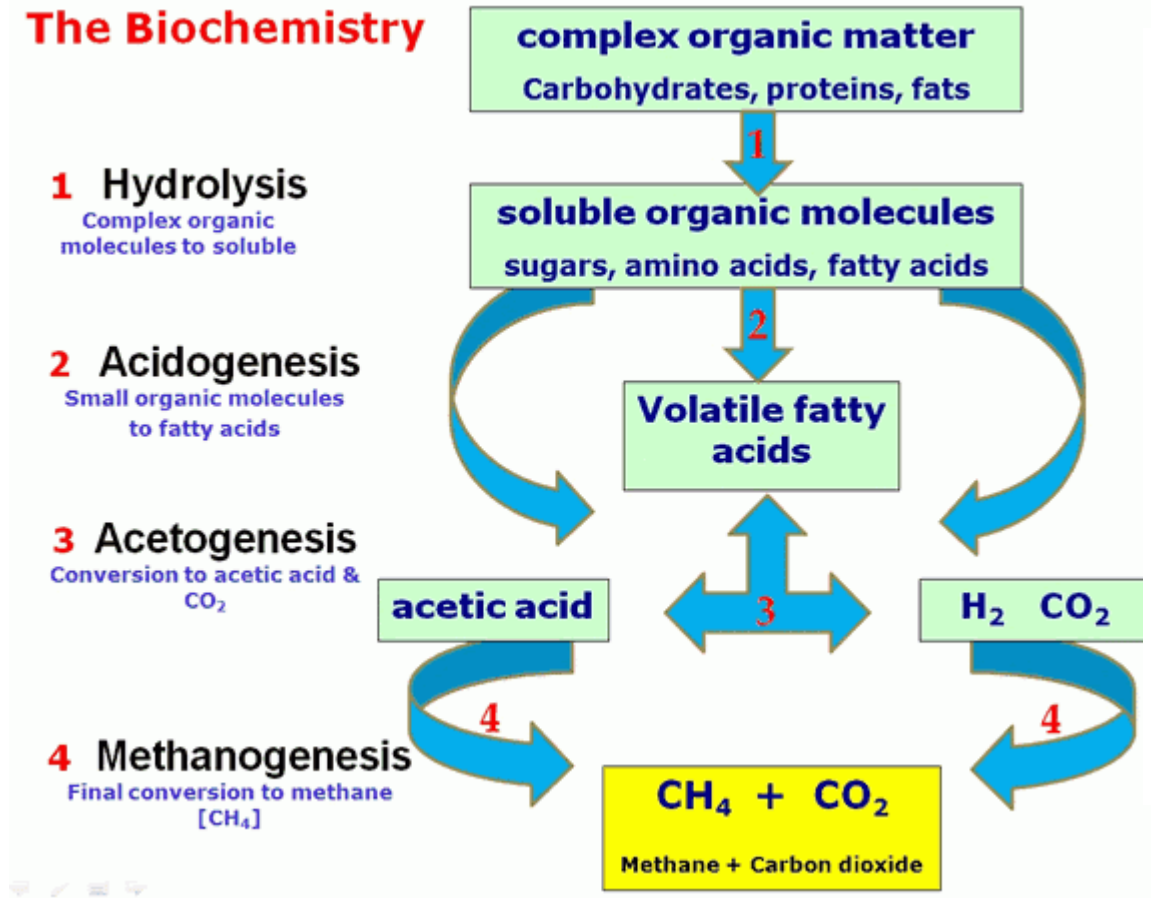
Microbial electrolytic cells

- high manufacturing costs of the cells
- high internal resistance
- issues due to biofouling

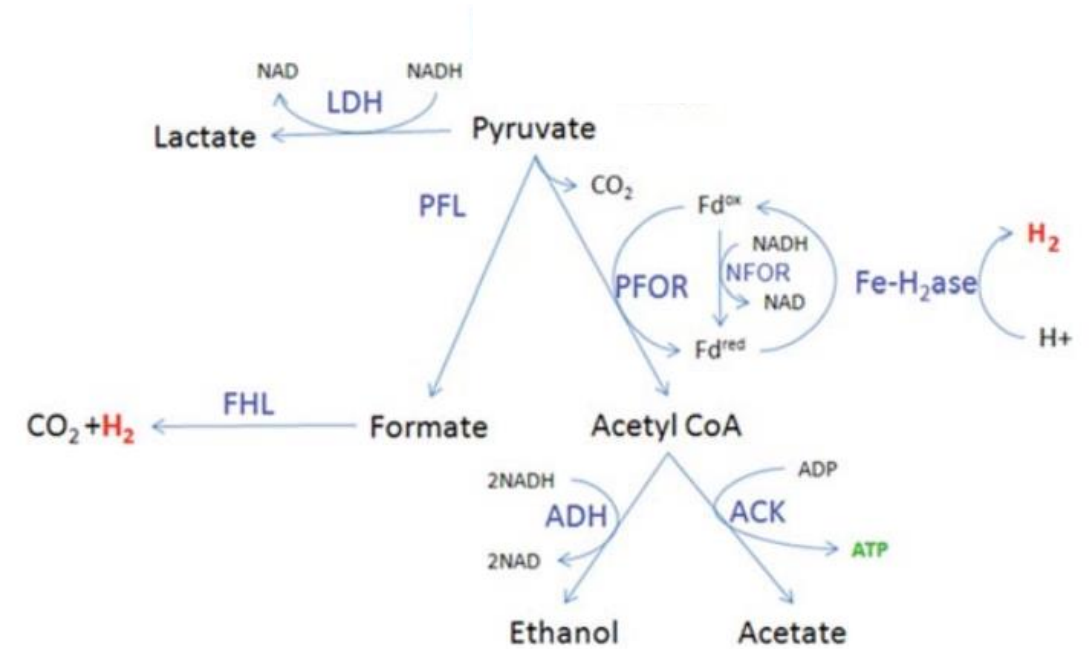
Anaerobic digestion phases



The Biochemistry



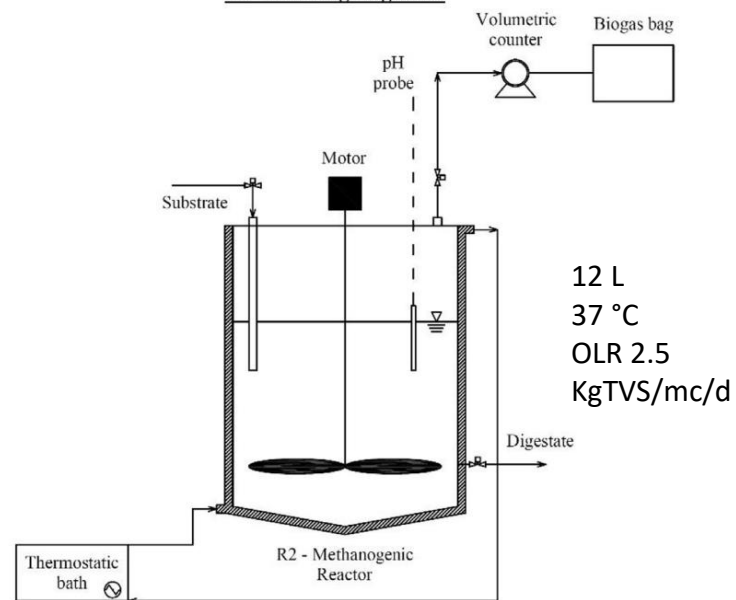
Dark Fermentation



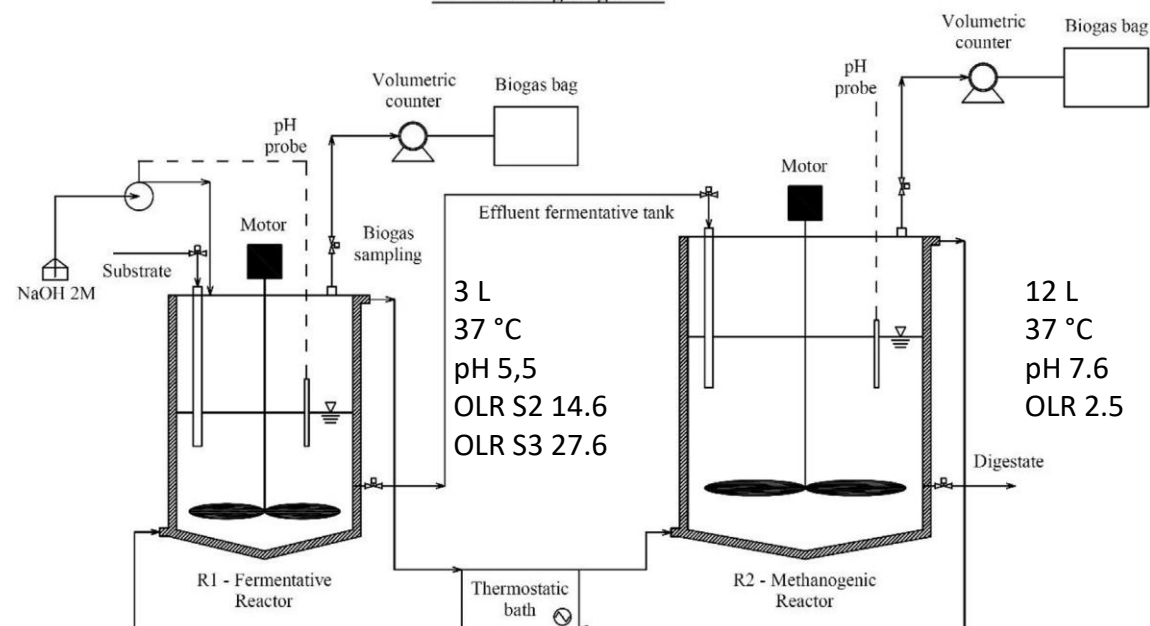
Bench scale reactors



1S - One-stage digestion



2S - Two-stage digestion



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FAS
Fondo Aree
Sottoutilizzate
2007-2013



REPUBBLICA ITALIANA

DF Start-up: easy way



FW:AS 1:5 (TS 5% c.ca)

Deaeration with Nitrogen

heat-shocked at 105 °C for 30 min

Acidic pH (5.5)



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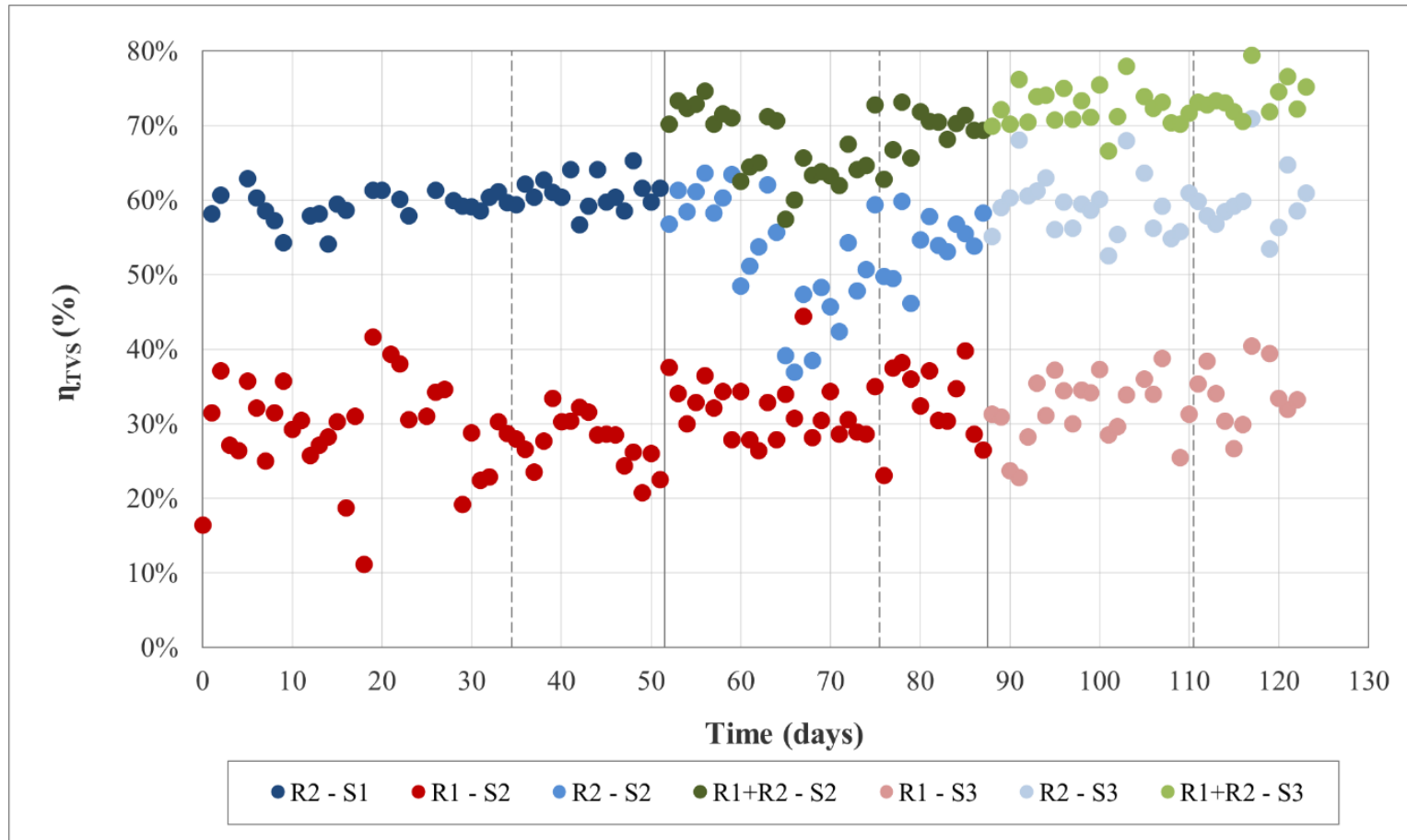


FAS
Fondo Aree
Sottoutilizzate
2007-2013



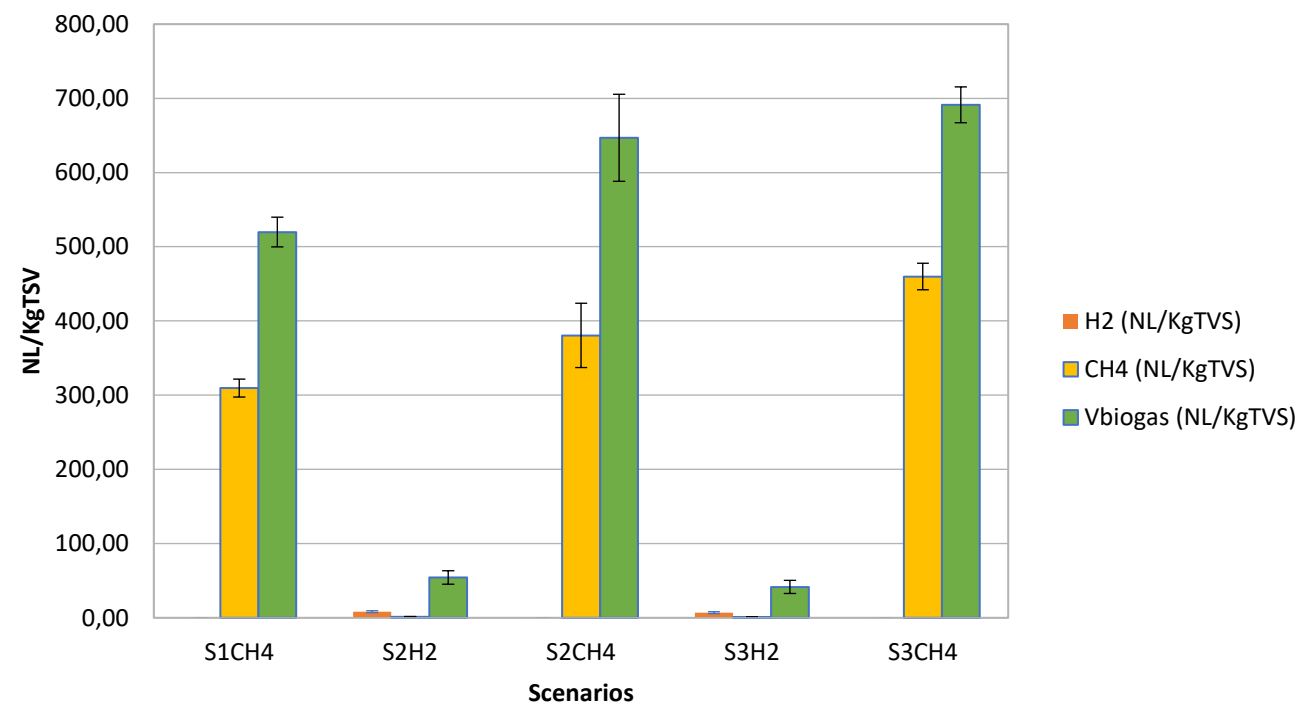
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Volatile solid removal efficiency



Red: Dark fermentation reactors
Blue: Anaerobic digestion reactors
Green: DF-AD in series

Biogas production



Very poor hydrogen production BUT
Methane production enhanced by
50% in scenario 3 (1,5 HRT)

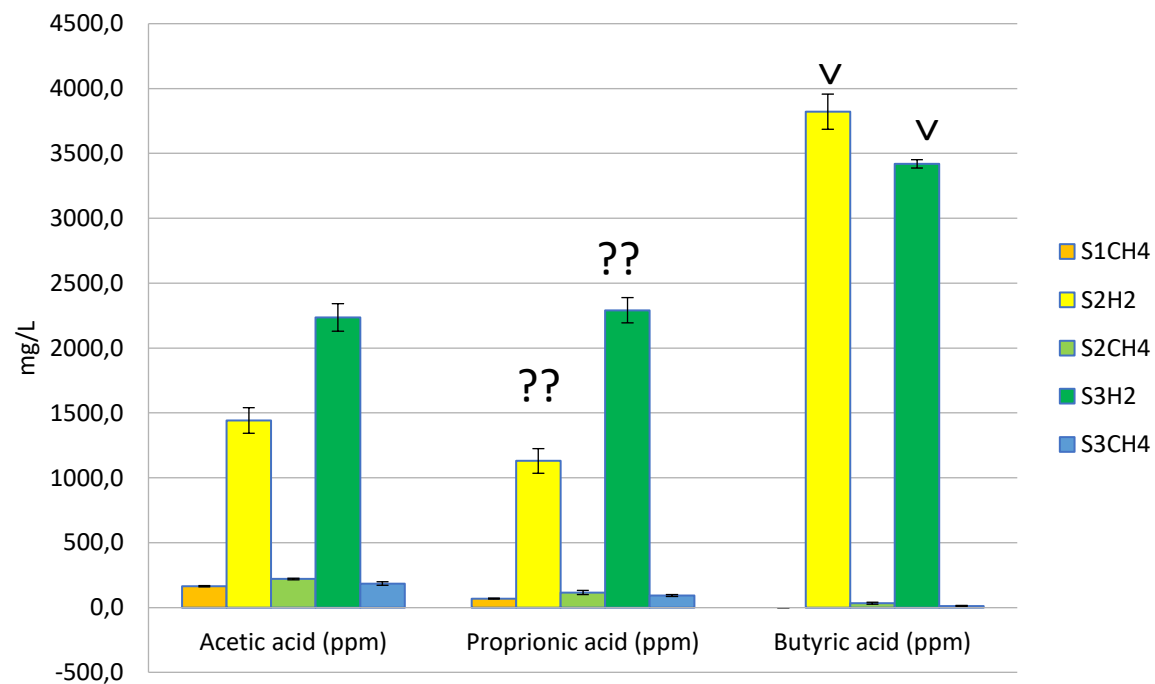
Scenario 2
111 NL/mc/d hydrogen

Scenario 3
274 NL/mc/d hydrogen

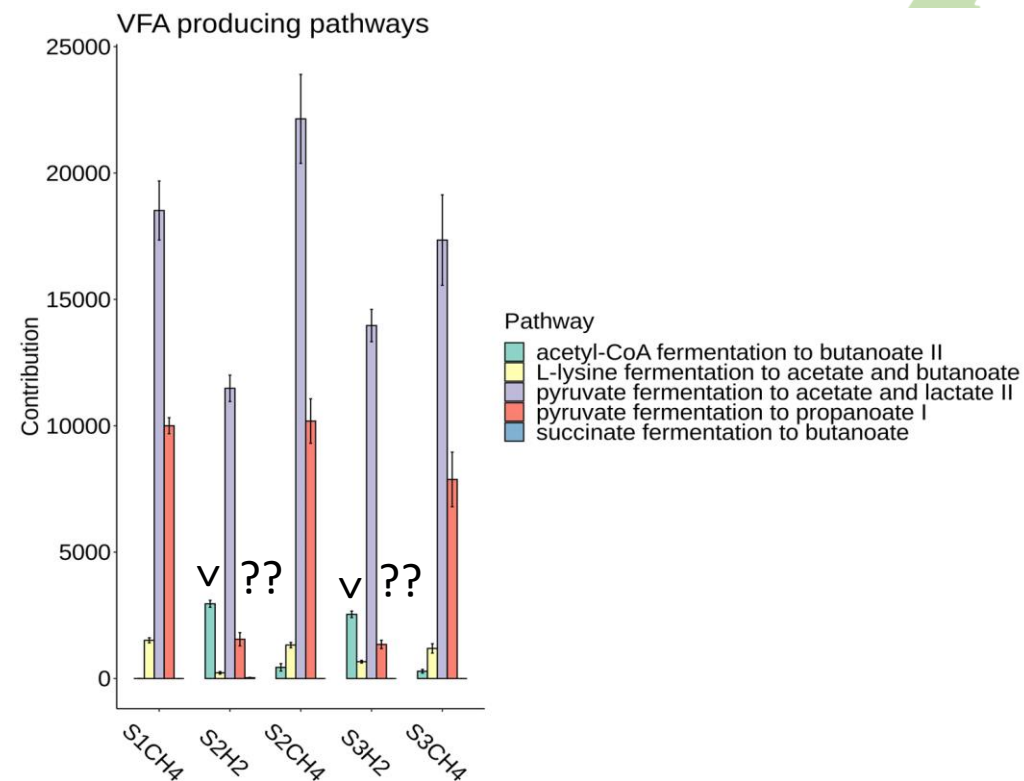
Optimised DF process
(glucose, pure culture)

OPTIMAL
640 NL/mc/d hydrogen

VFA production

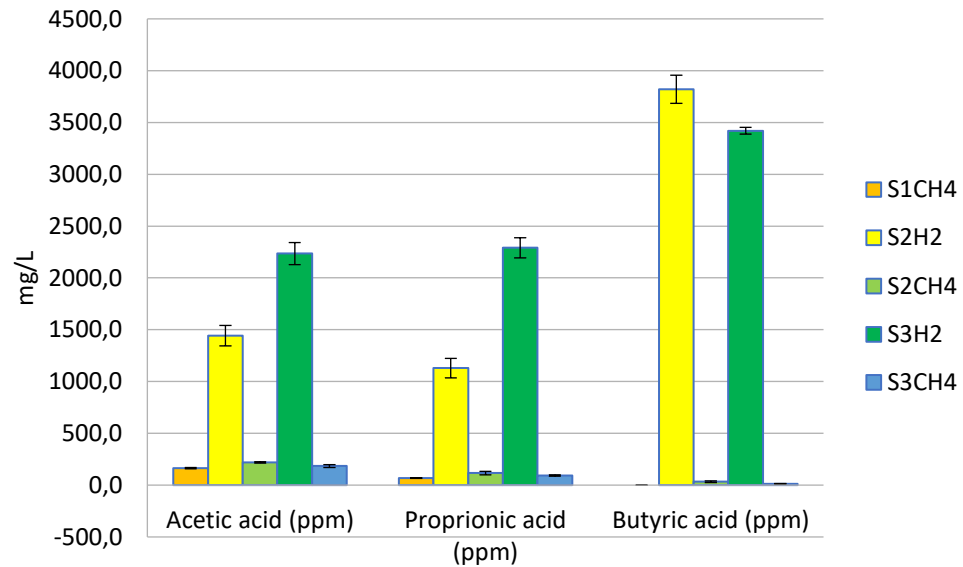


Butanoate production is higher in hydrogen sectors
But propanoate production is lower



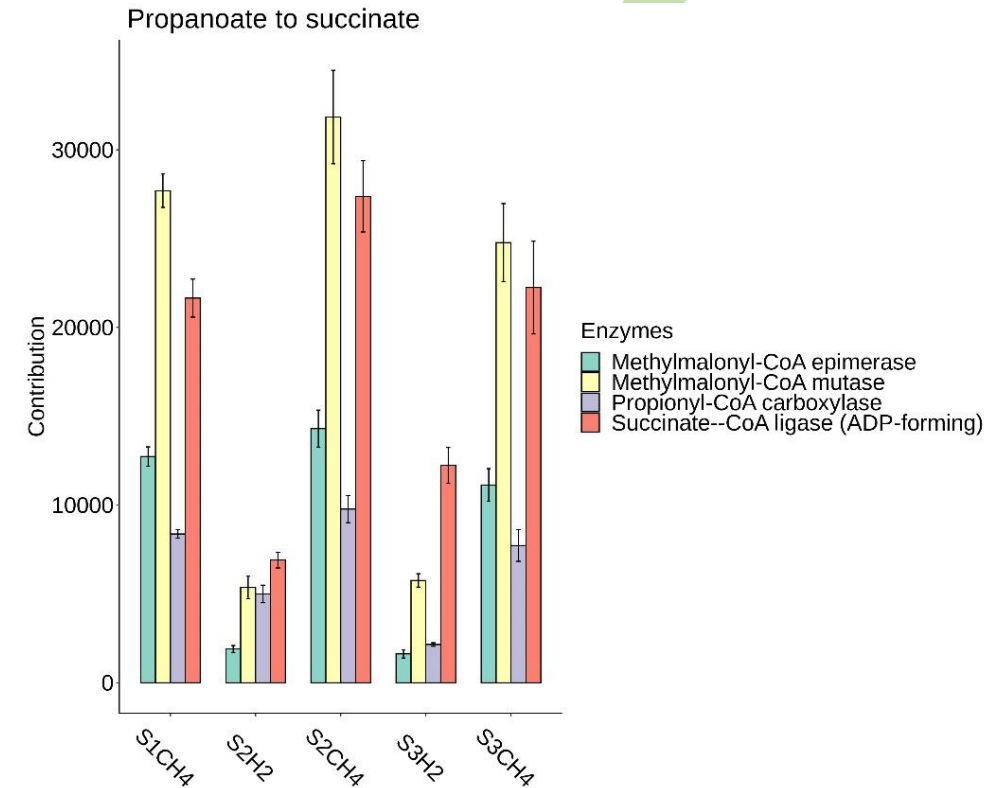
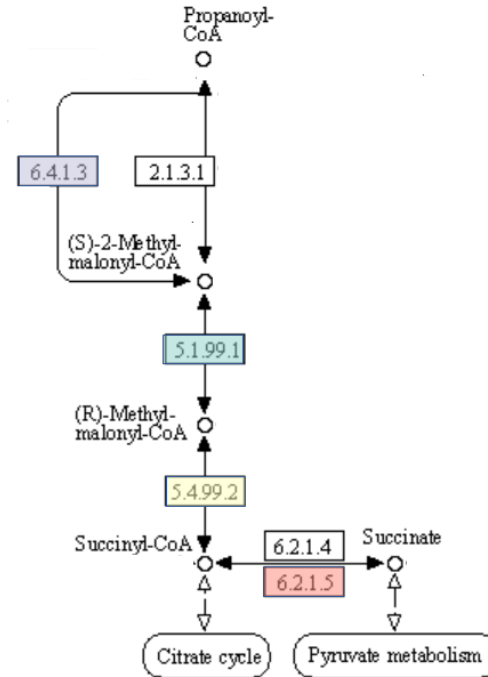
Succinate goes to
pyruvate metabolism

Propanoate consumption



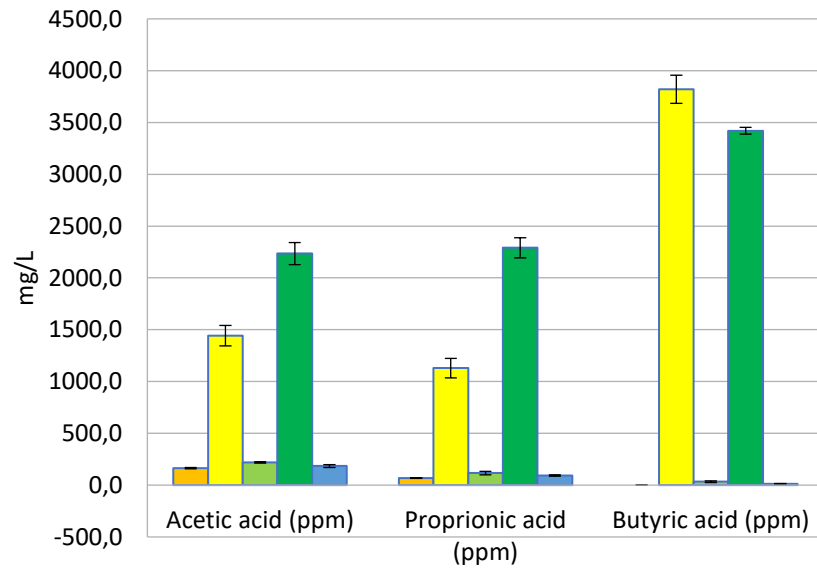
Microbial potential for propanoate metabolism is higher in methane producing sectors

ACCUMULATION EFFECT in hydrogen sectors

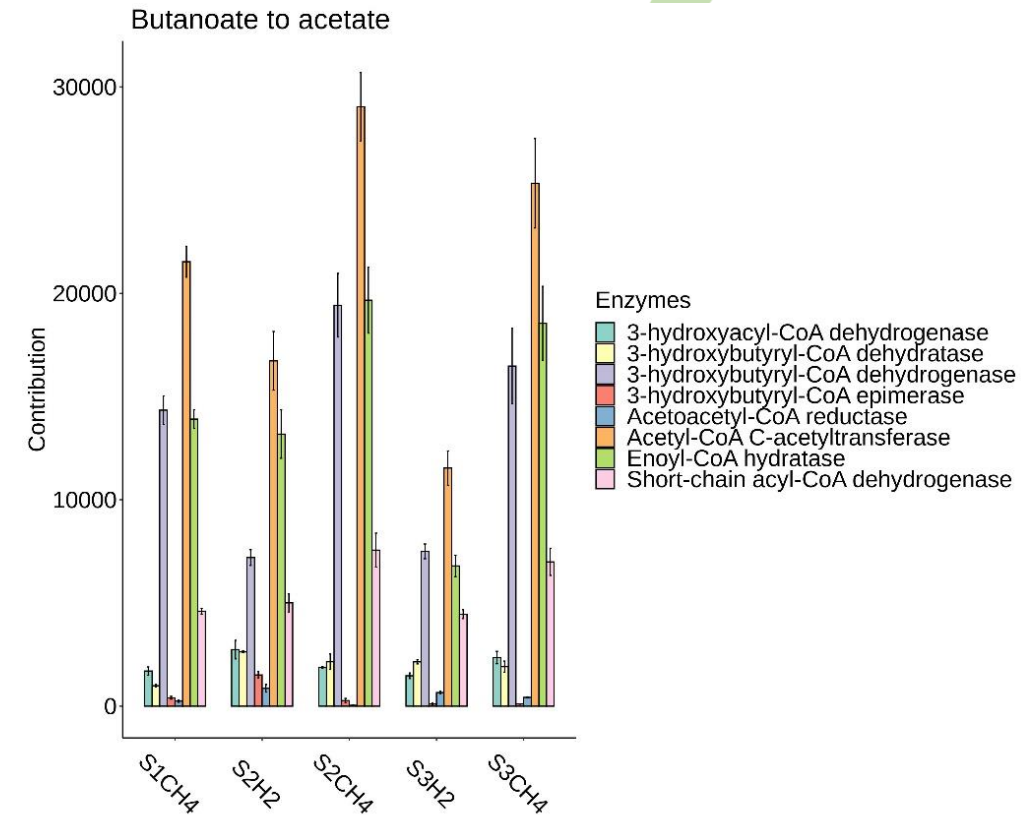
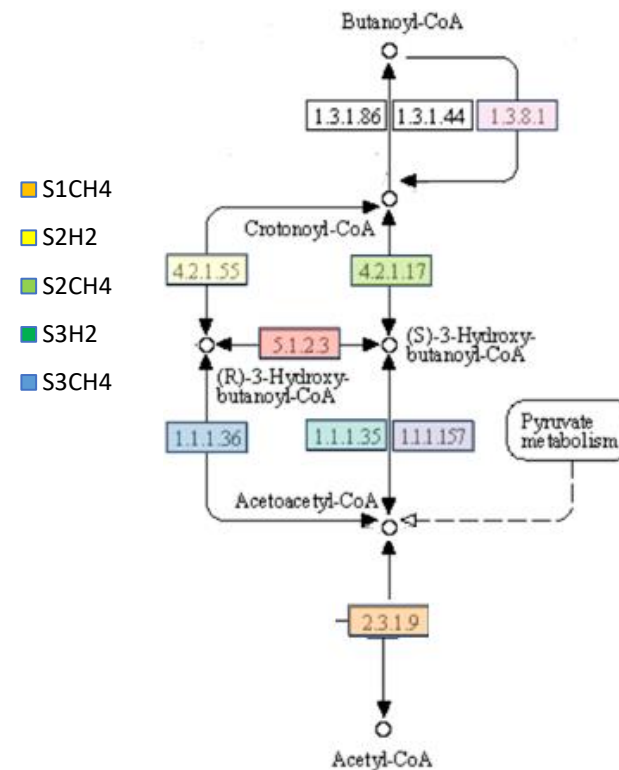


Succinate goes to pyruvate metabolism

Butyrate consumption



Butyrate consumption is slightly faster at lower HRT

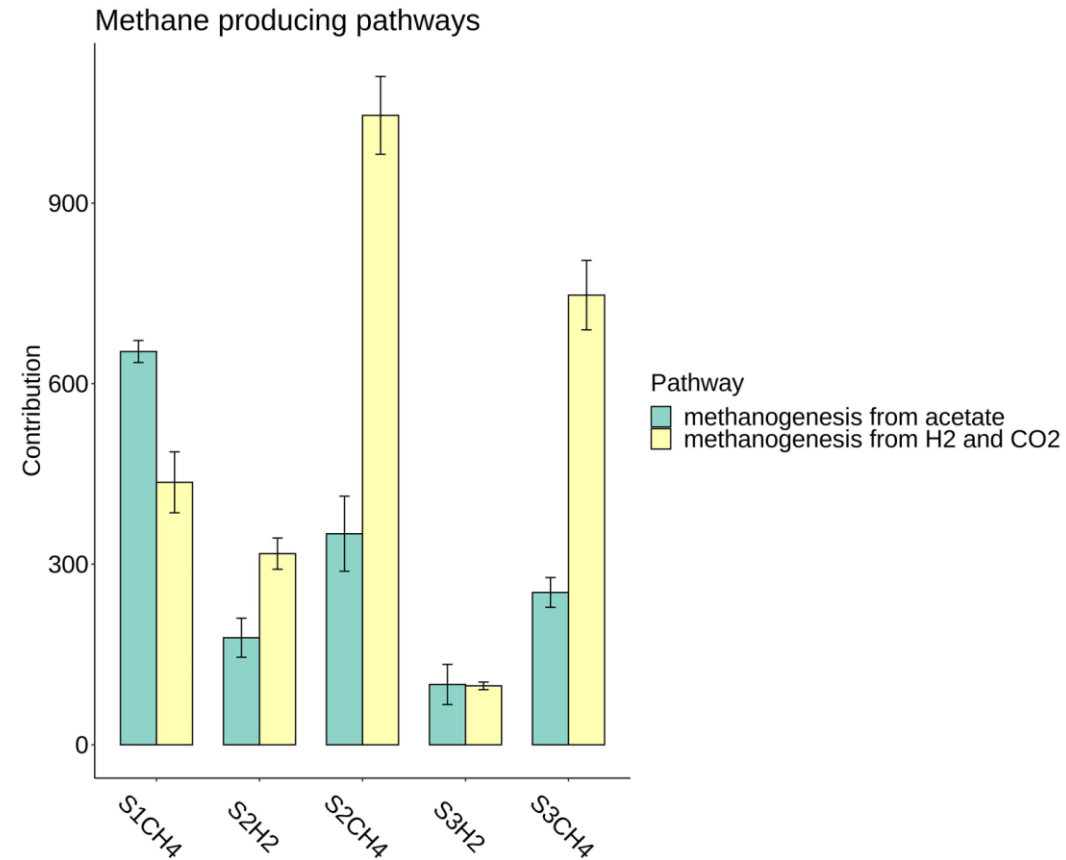


Acetoclastic v.s. hydrogenotrophic

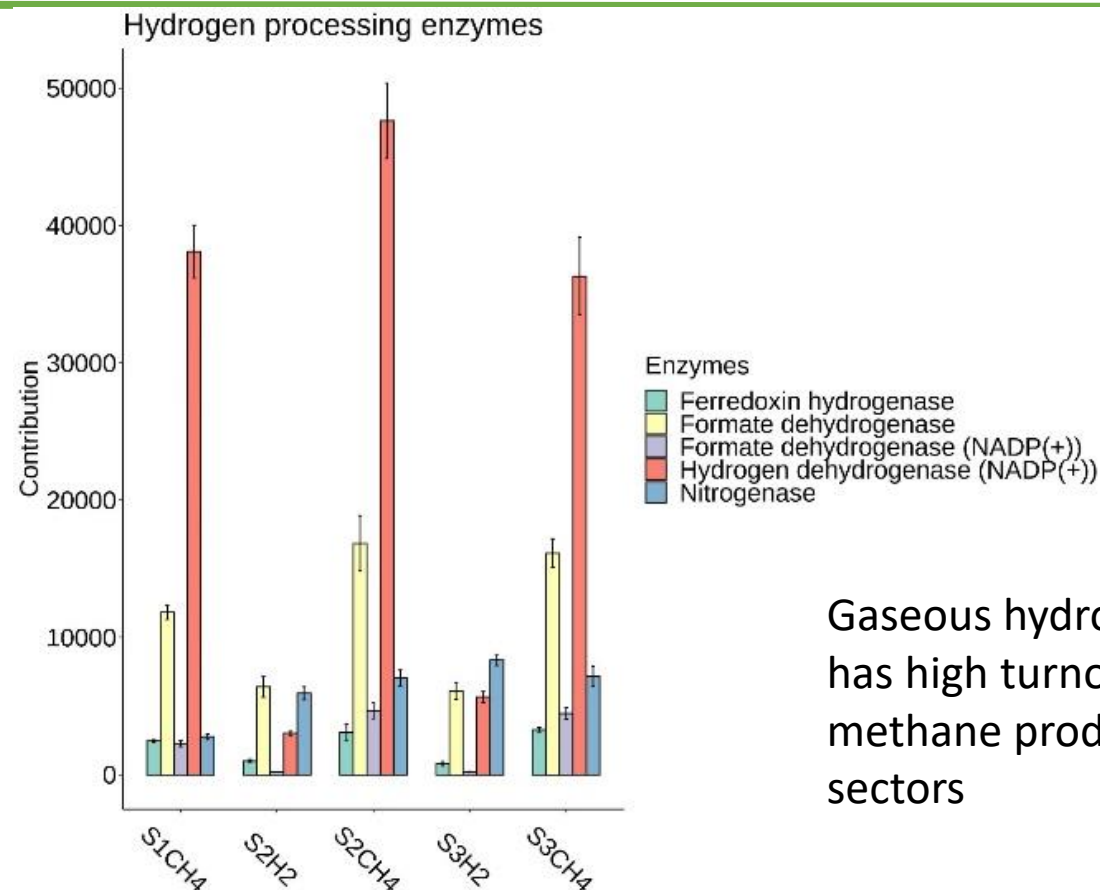


	Archaea (%)	SEM (%)
S1CH ₄	3,25	0,23
S2H ₂	1,62	0,13
S2CH ₄	4,87	0,61
S3H ₂	0,80	0,04
S3CH ₄	5,45	0,17

Metabolic shift toward
hydrogenoclastic
methanogens



Hydrogen processing enzymes inference



Gaseous hydrogen has high turnover in methane producing sectors

Nitrogen fixation is the main responsible for direct hydrogen production

Ferredoxin hydrogenase is the second contributor

Formate is the inter-species hydrogen transporter



THANK YOU for your kind attention



AND ALSO THANKS TO

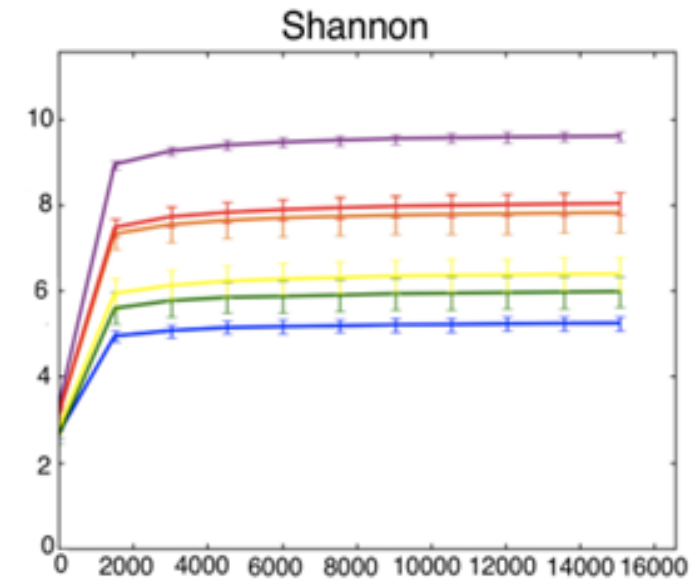
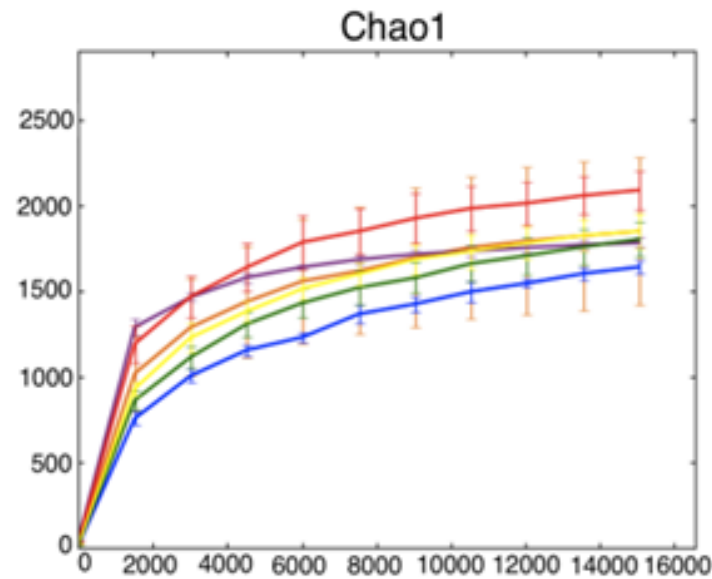
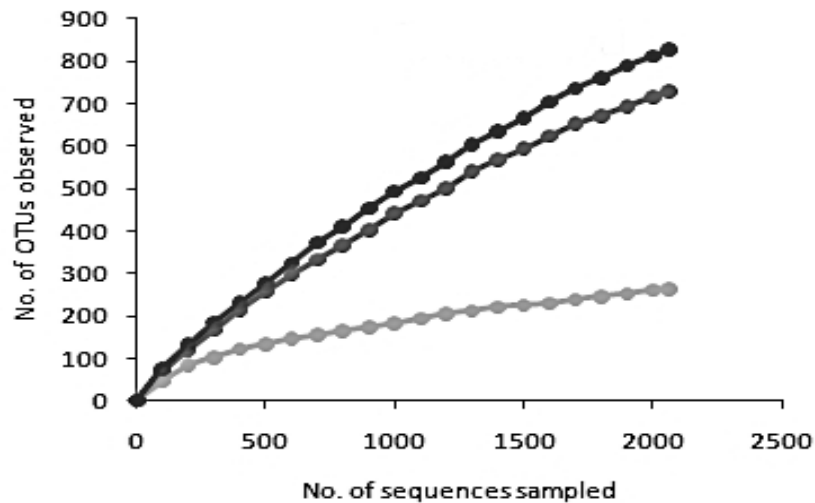
Elena Rossi,

Isabella Pecorini,

Simona di Gregorio,

Renato Iannelli

Alfa diversity



Legend

- B T0
- B T60
- Baut T0
- Baut T60
- notB T0
- notB T60

Stimare la ricchezza e l'equidistribuzione delle unità tassonomiche nei campioni e valutarne la differenza tra campione e campione

- Rarefazione ad **un numero comune di OTU per ogni campione**
- Chao1 index
- Shannon (Hill $\lim_{q=1}$)
- Simpson (Hill $q=2$)

$${}^qD = \left(\sum_{i=1}^S p_i^q \right)^{1/(1-q)}$$

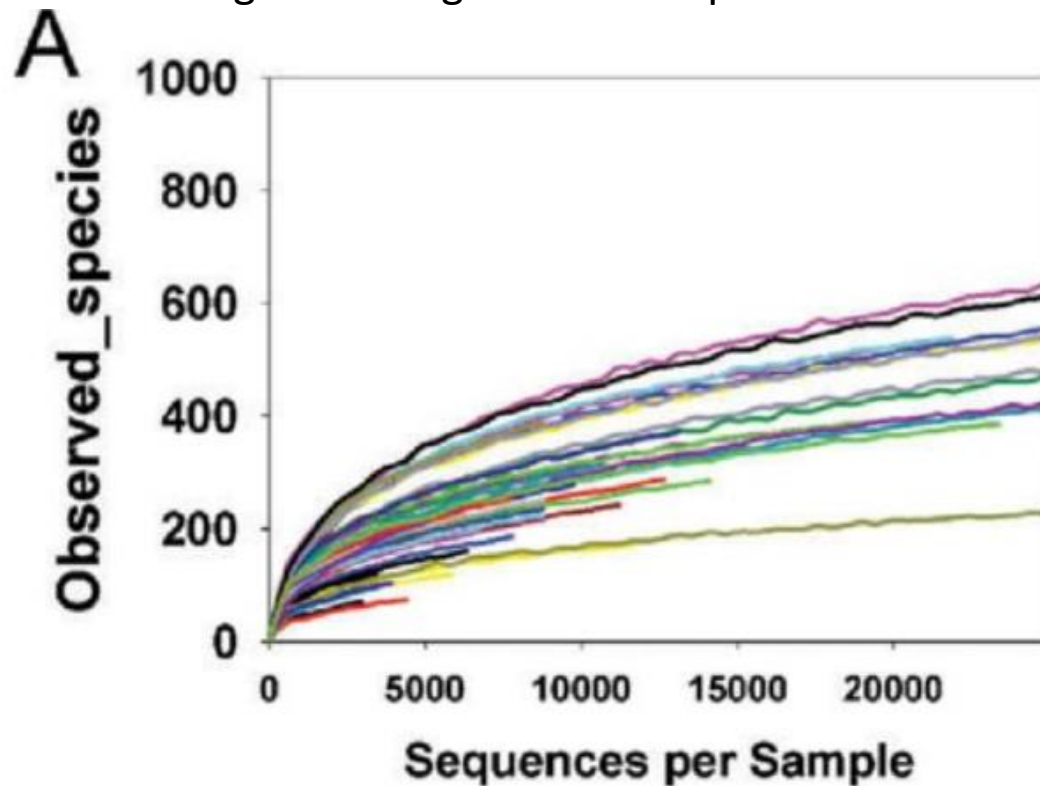
Test generale Kruskal Wallis
Post hoc : Dunn test
correzione confronti multipli:
Benjamini-Hochberg FDR

E statistica (senza statistica un dato non è rispettabile)

MA SPESSO E VOLONTIERI

Alcuni campioni hanno un numero di sequenze estremamente inferiore agli altri

Dover «gettare» il grosso del sequenziamento è ABOMINEVOLE



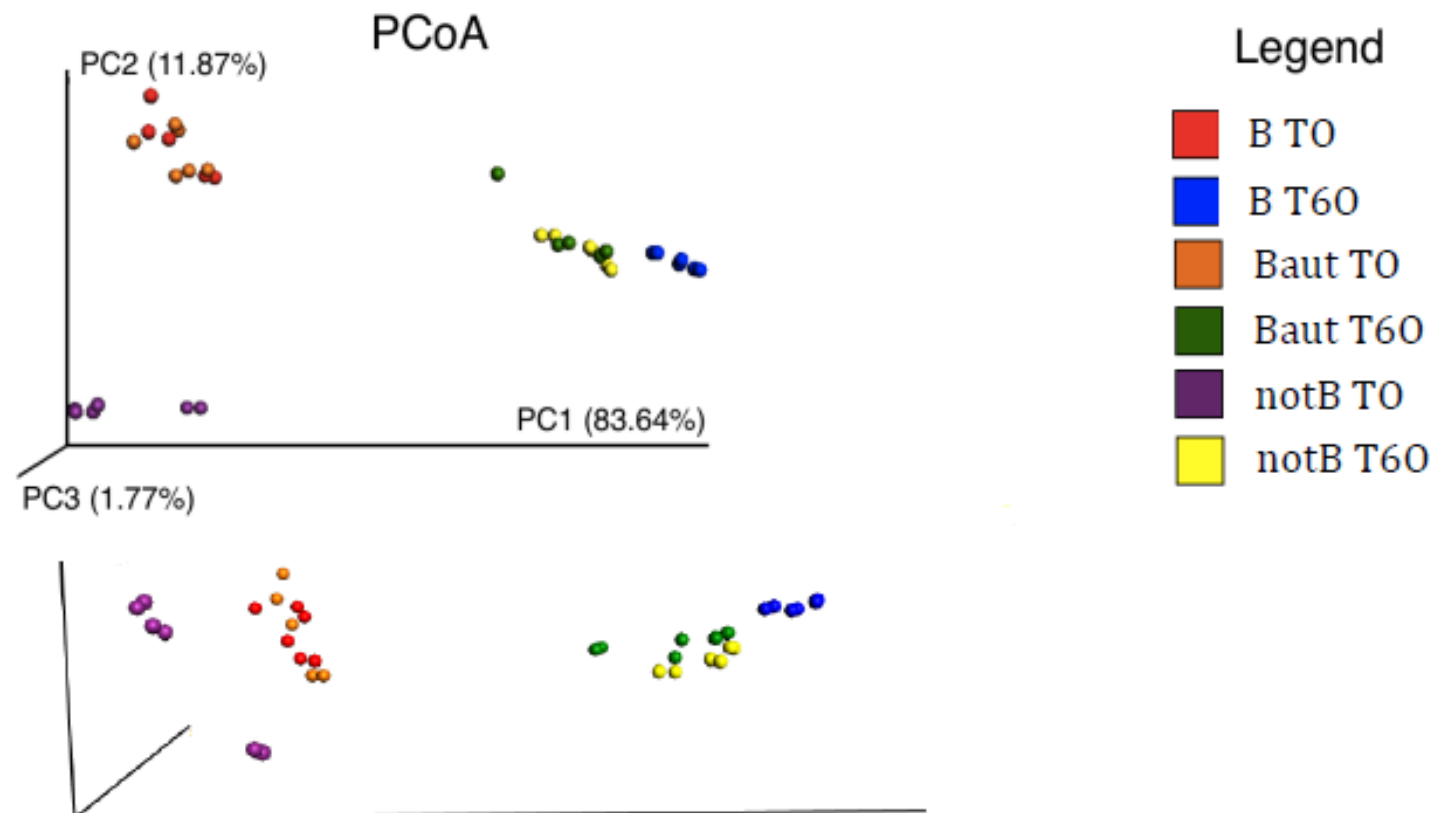
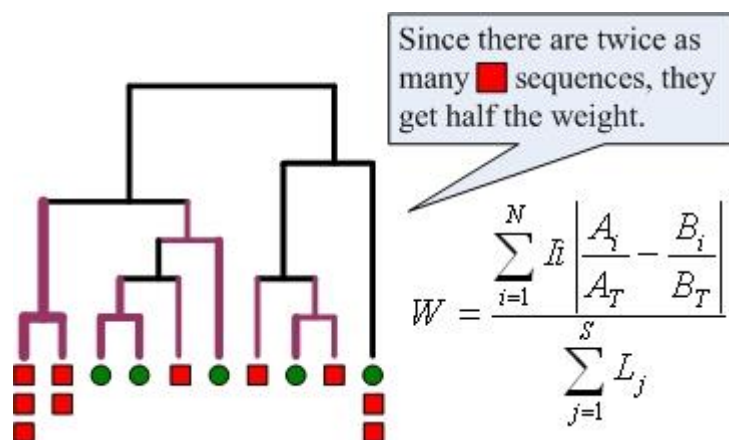
Allora si usa una rarefazione basata sul coverage

e.g. si confrontano i valori di richness e evenness al 99,5% di taxa individuati

Molto dispendioso in termini di calcolo

Beta diversity

Quantifica la «distanza» nella composizione (e abbondanza) della comunità microbica tra «ambienti diversi» (gruppi di replicati) facendone una combinazione lineare

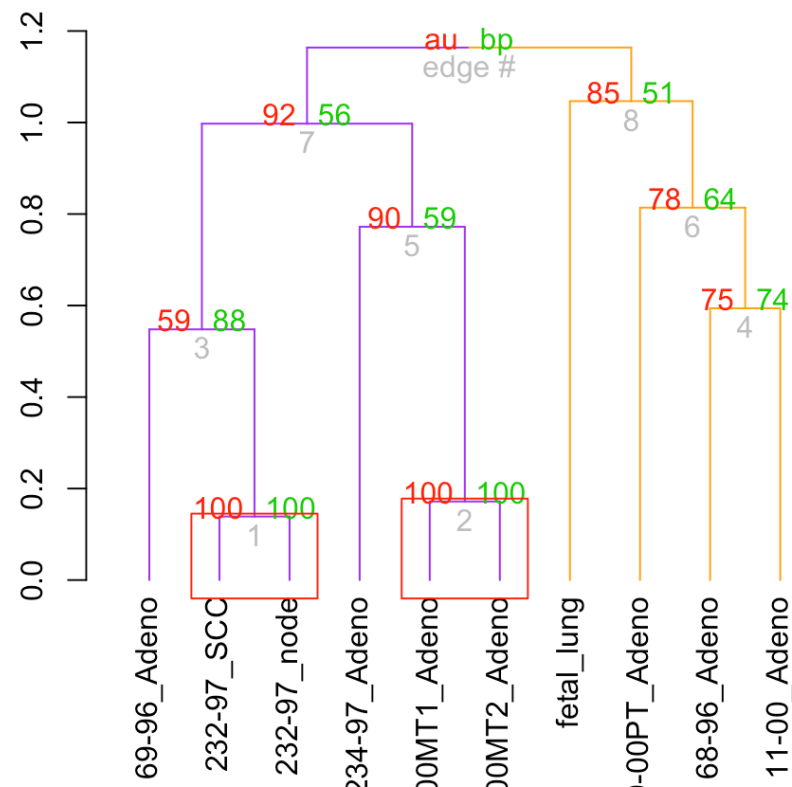


La rappresentazione è una **Principal coordinate Analysis** e la statistica è la PERMANOVA con 999 reiterazioni

La statistica per la Beta diversity

Basata su la PERMANOVA

Ogni cluster è ricalcolato assegnando una comunità microbica ad un cluster di similarità differente, e si calcola un indice di stabilità (BOOTSTRAP)





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Metagenomics and metabarcoding approaches to describe ecological systems and infer their development

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Enhanced biogas production from sulfate rich wastewater generated from concentrated latex factory

Prawit



European
Commission



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