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## Characterization of virus-host dynamics in anaerobic digesters under abiotic stress

Marion Coves, Laurent Mazéas, Olivier Chapleur, Cédric Midoux, Ludwig E Jardillier, Ariane Bize

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Société Française  
de Microbiologie

## ➤ Characterization of virus-host dynamics in anaerobic digesters under abiotic stress

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**PROSe**  
PRocédés biOtechnologiques  
au Service de l'Environnement

 **MaIAGE**  
MATHÉMATIQUES ET INFORMATIQUE APPLIQUÉS DU GÉNOME À L'ENVIRONNEMENT

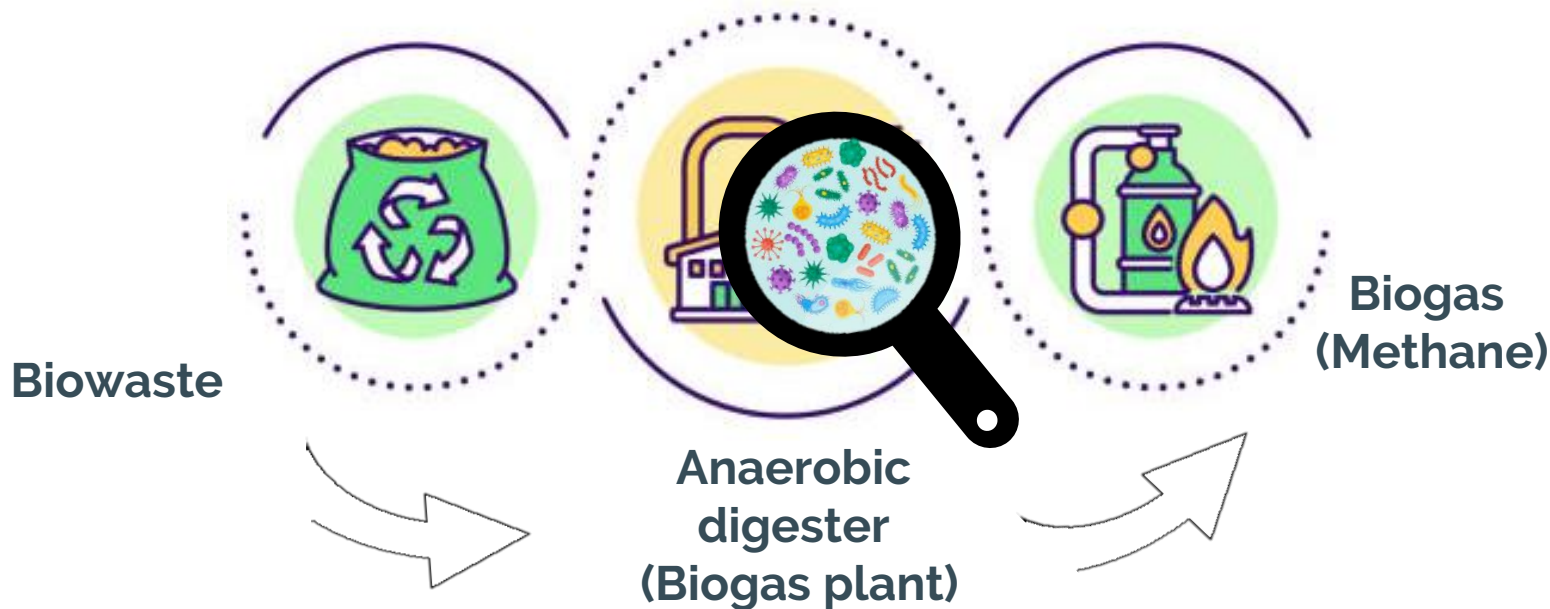
Ecole Doctorale  
**ABIES**  
Agriculture  
Agriculture  
Alimentation  
Food  
Biologie  
Biologie  
Environnement  
Environnement  
Santé  
Santé

AgroParisTech  
Talents d'une planète soutenable 

ANR-17-CE05-0011

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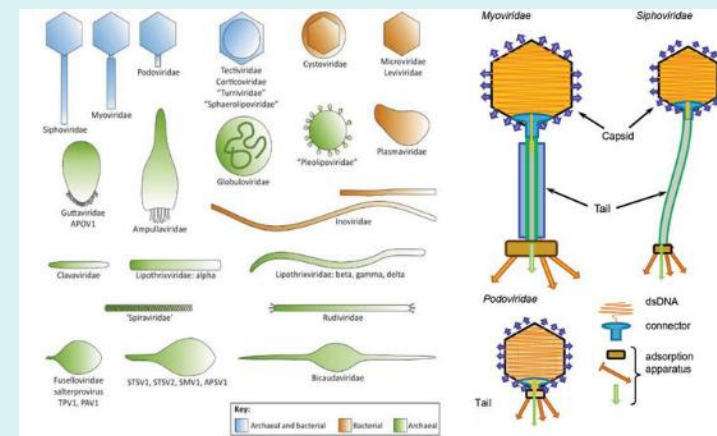
# Characterization of virus-host dynamics in anaerobic digesters under abiotic stress



**=**  
**Complex ecosystem**  
**very sensitive to**  
**perturbation**

Essential role of Microorganisms

What about **viruses** of microorganisms ?



White, et al. 2019

Impact on microbial communities and biogeochemical cycles demonstrated!

Weitz, et al. 2012

# ➤ Are there viruses of microbes in anaerobic digesters ?

YES, they are:

- Numerous
- Diverse in shapes, sizes, genome content...
- Almost only DNA viruses
- Poorly studied

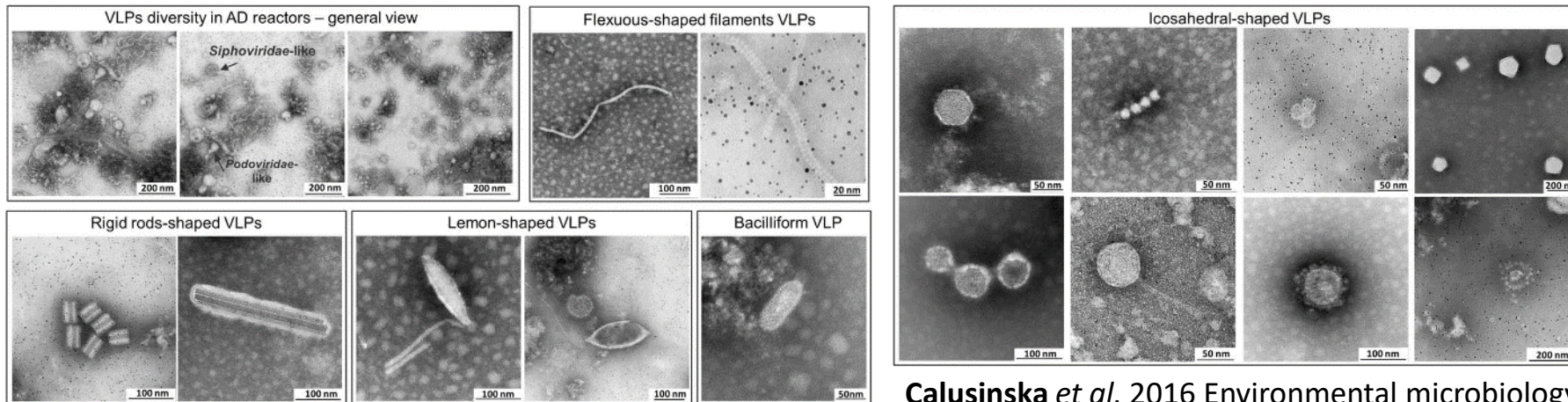
Statement: **Inhibition can impact the microbial community directly and indirectly.**

Question: **Is the viral community one of the indirect factors ?**

Hypothesis: **Stress may activate the lytic cycle of temperate viruses, causing cell death increase and thus more inhibition.**

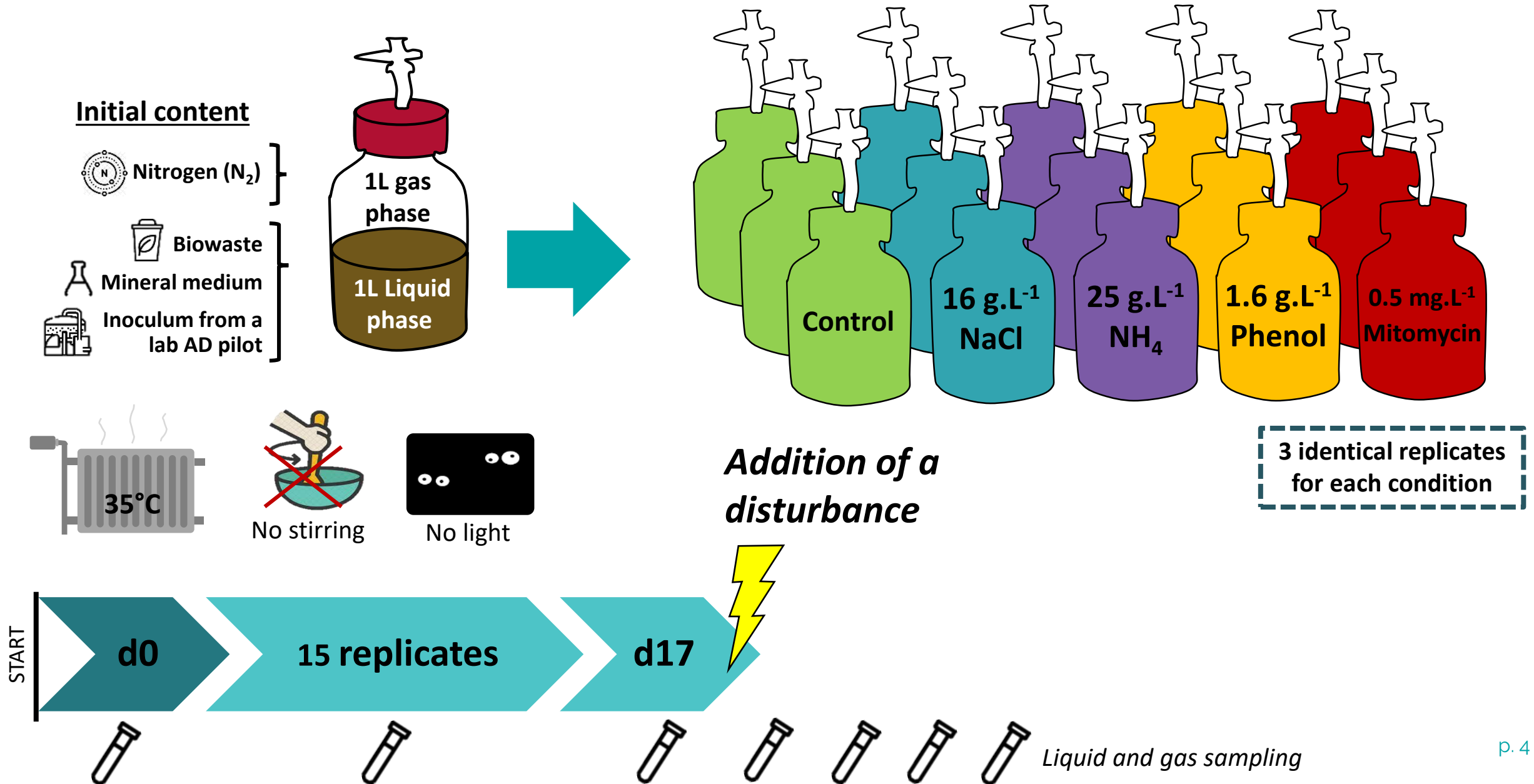
“molecular time bombs”, Paul, *et al.* 2008

## Diversity of viral structures in anaerobic digesters

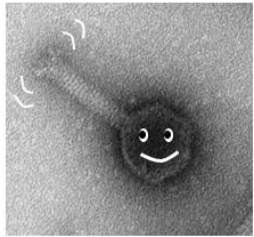


Calusinska *et al*, 2016 *Environmental microbiology*

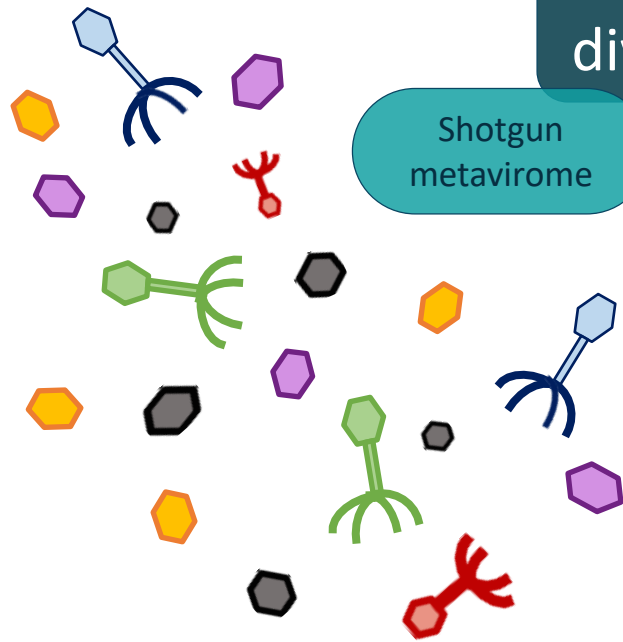
# ➤ Experimental design



# ➤ Experimental design



(Non-contractual picture)



Electron microscopy

Viral diversity

Shotgun metavirome

Global dynamics

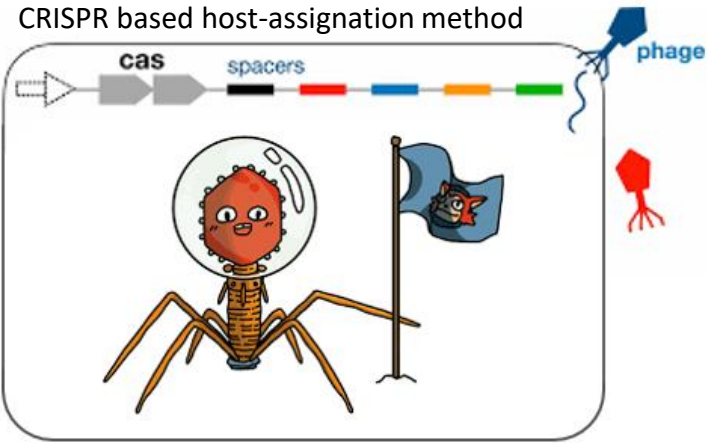
Biogas production, pH, metabolic intermediates (Volatile Fatty Acids)

Prokaryotic diversity

ADNr 16S Metabarcoding

Virus-Host interaction and ecosystem functioning

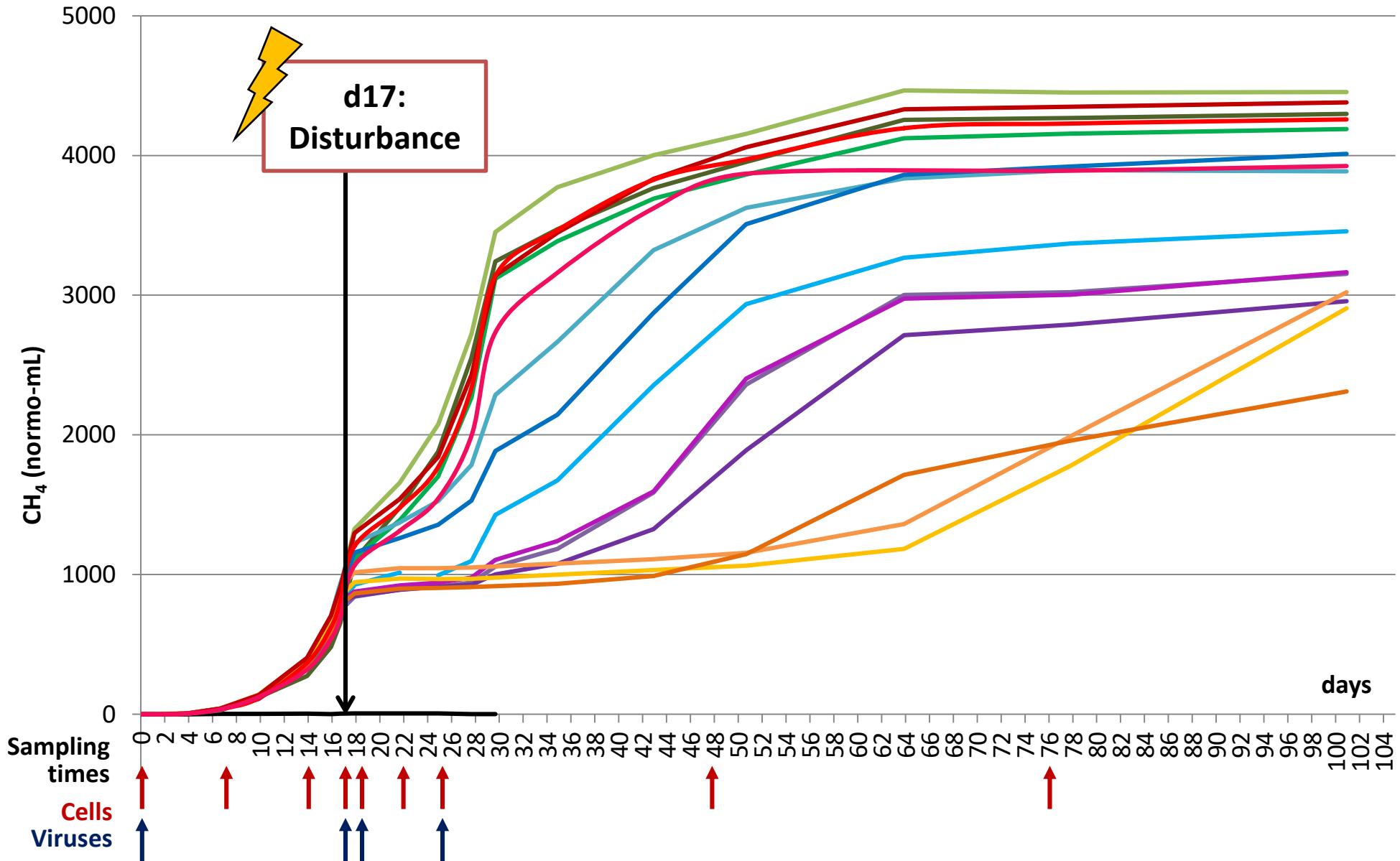
Bioinformatics approaches



Ruoshi Zhang, et al. 2021, SpacePHARER



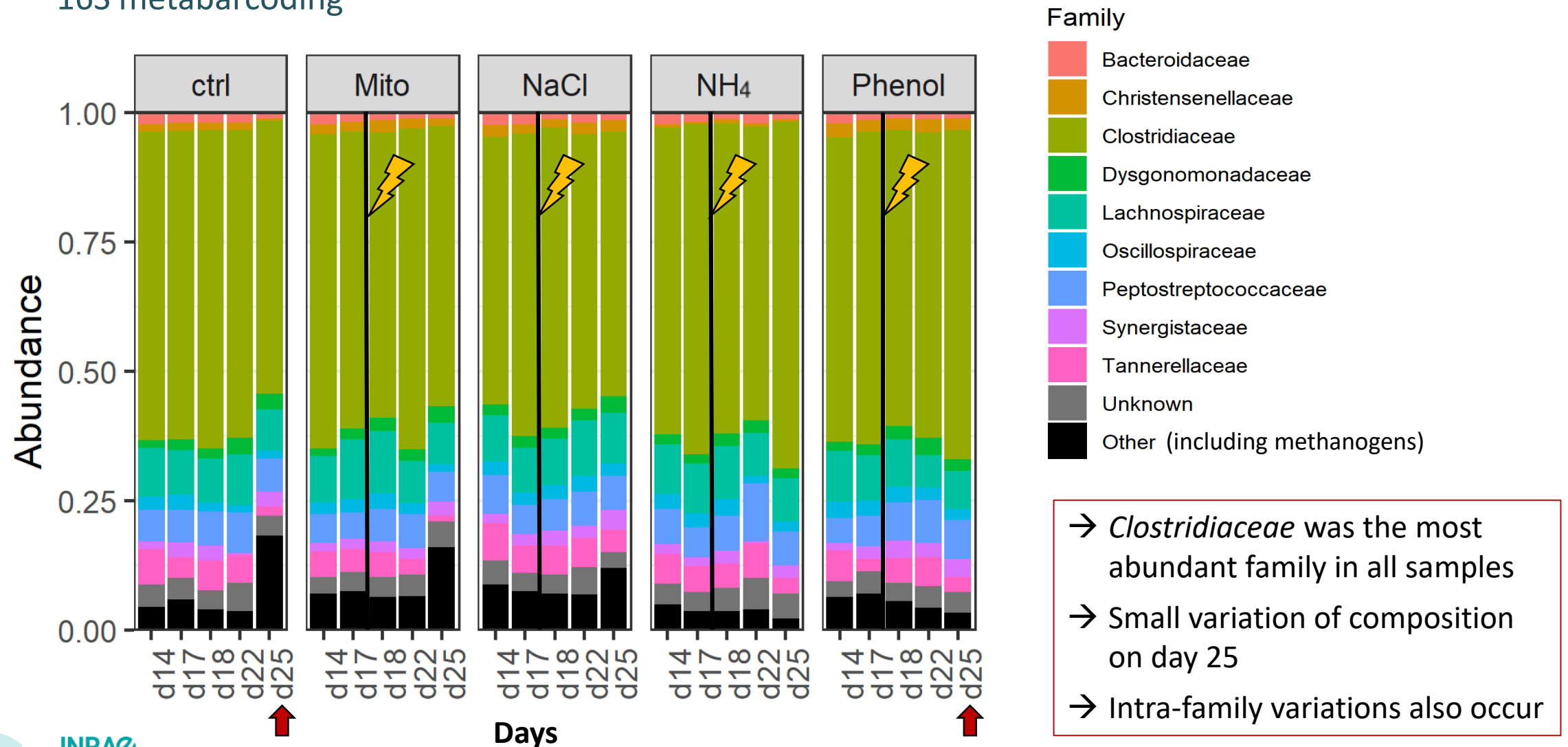
# ➤ Impact on CH<sub>4</sub> cumulative production?



NaCl, NH<sub>4</sub> and Phenol conditions are impacted !

# ➤ Impact on microbial community composition at the family level?

16S metabarcoding



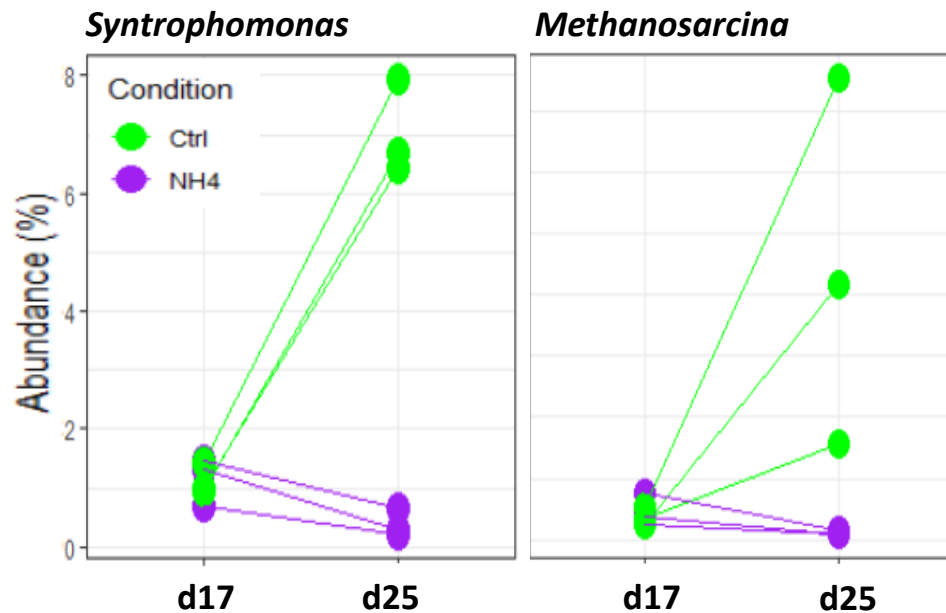


# ➤ How many genera are affected by the disturbances?

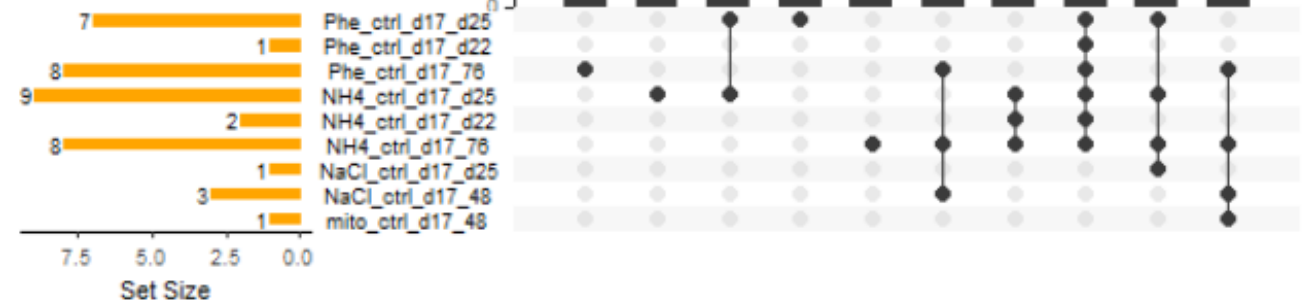
Differential abundance analysis

Differentially abundant genera between :

- **Control and disturbed** condition
- from **day before to day after** disturbance



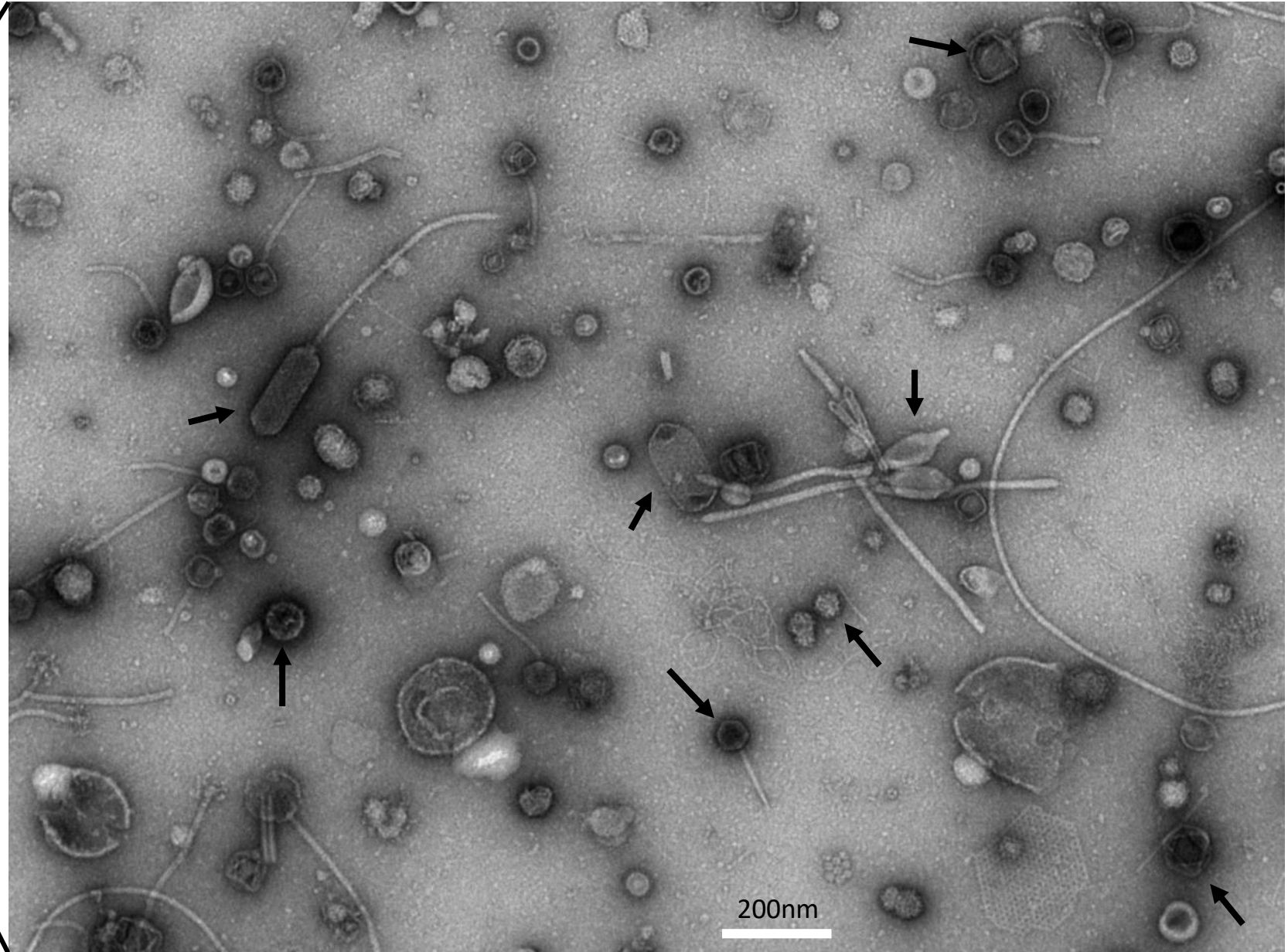
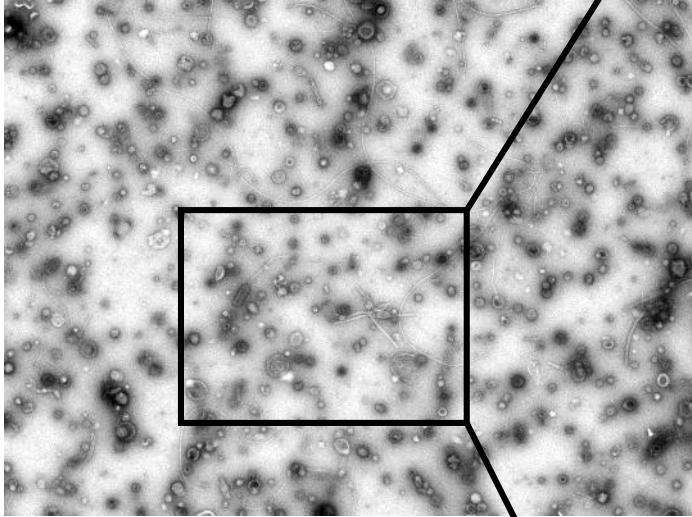
Hits of differentially abundant genera sorted by condition and times



- ➔ *Syntrophomonas* and *Methanosarcina* genera abundance evolutions are consistent with biogas production inhibition
- ➔ 20 distinct genera observed significantly different dynamics
- ➔ 5 are common to the three most inhibited conditions with NaCl, NH<sub>4</sub>, Phenol

## ➤ Viruses, Are you here?

Electron microscopy



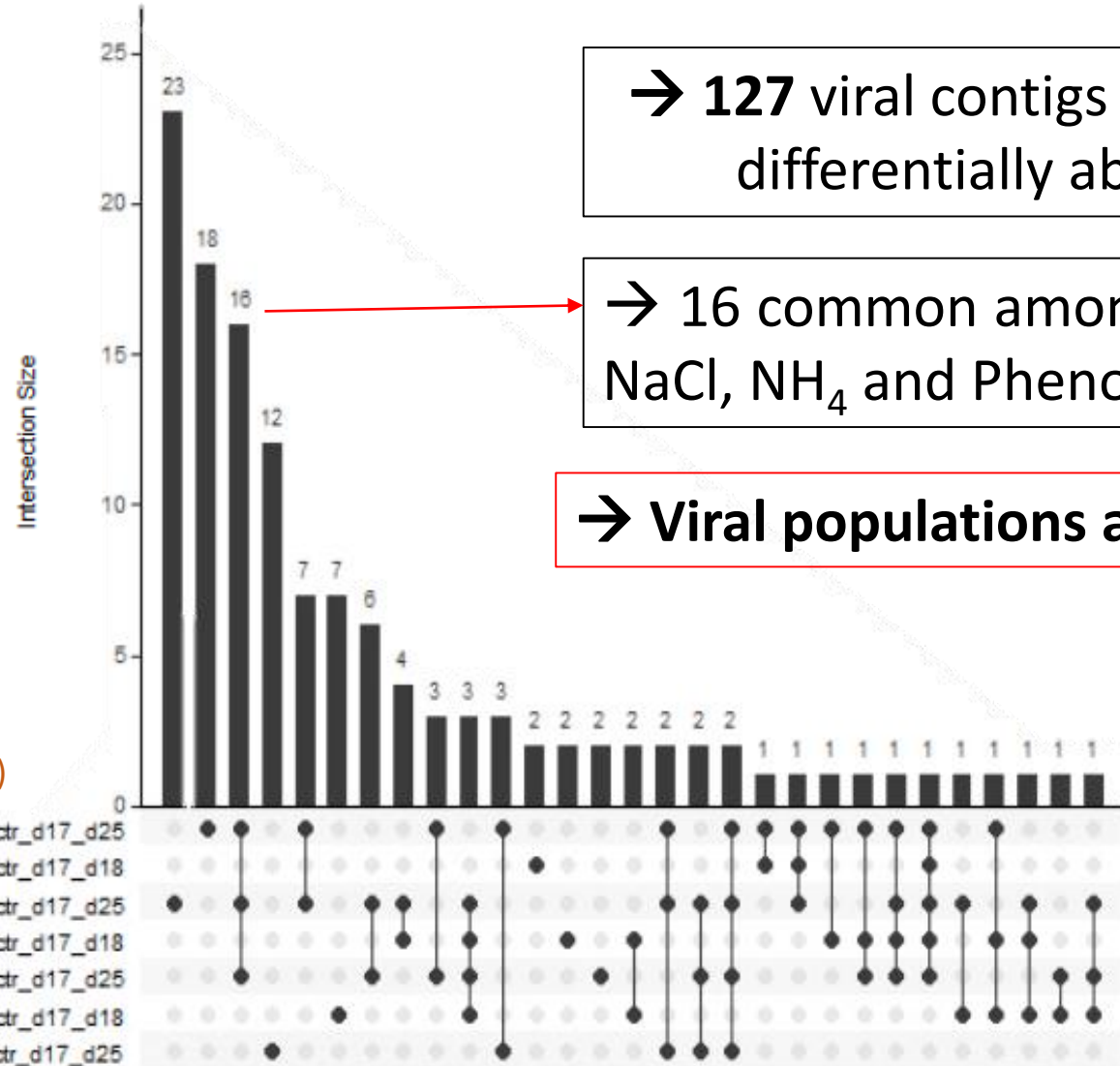
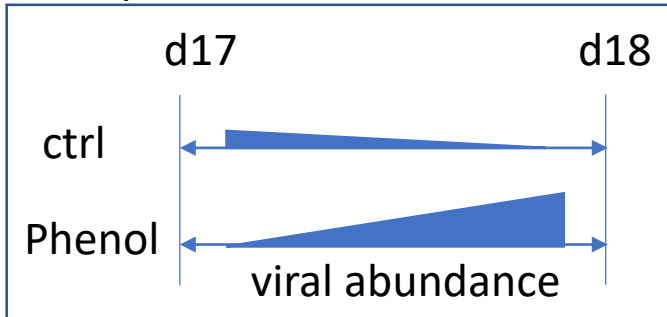
- Viral particles isolated by filtration (0.22 $\mu$ m)
- 690ng per sample of DNA extracted in average

→ Viral particles are numerous and diverse in shapes and sizes

# ➤ How many viruses are affected by the disturbances ?

Differential abundance analysis

example

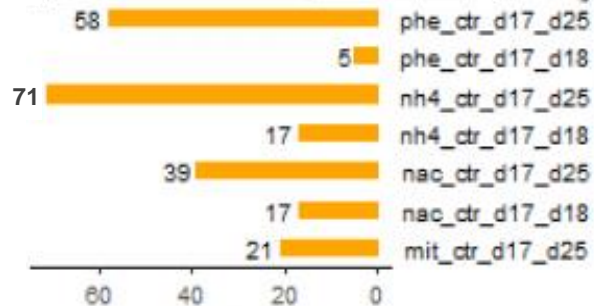


➔ 127 viral contigs (out of 430) differentially abundant

➔ 16 common among conditions NaCl, NH<sub>4</sub> and Phenol on day 25

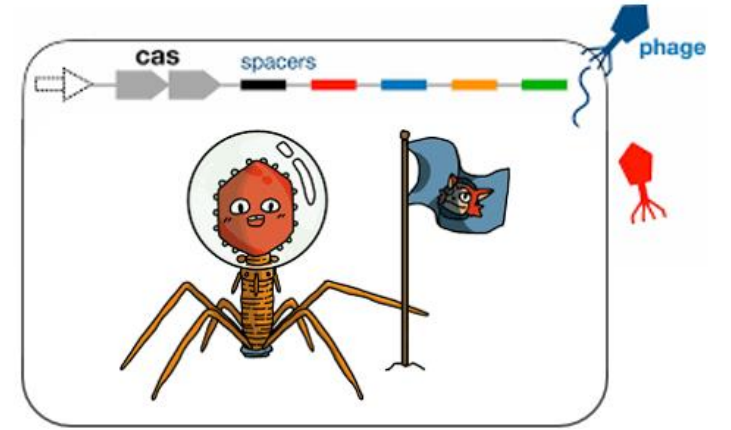
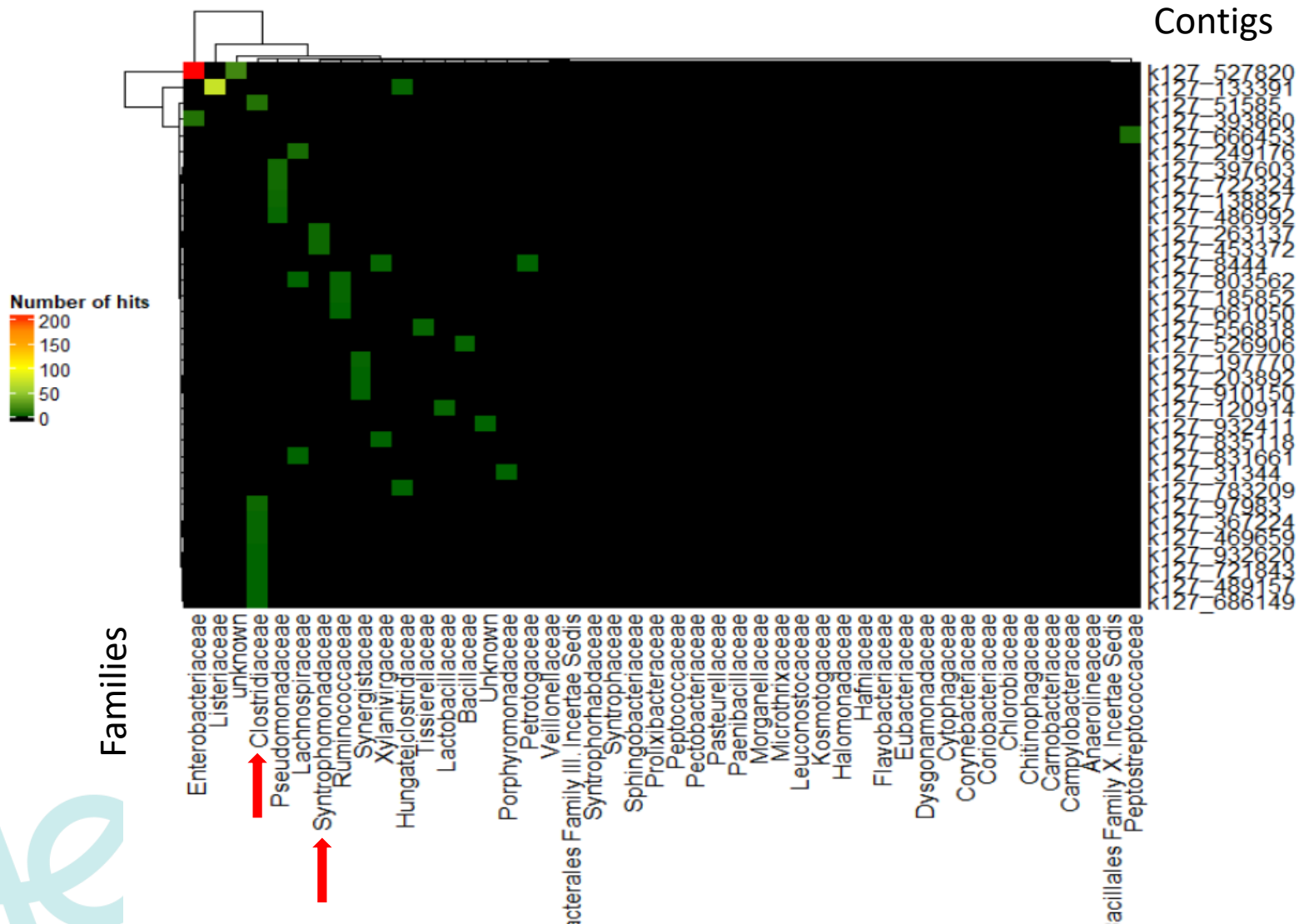
➔ Viral populations are impacted

Hits for differentially abundant contigs (by condition and times)



# Who are the hosts of these viruses?

CRISPR-spacer based host-assignment method (SpacePharer)



Ruoshi Zhang, et al. 2021, SpacePHARER

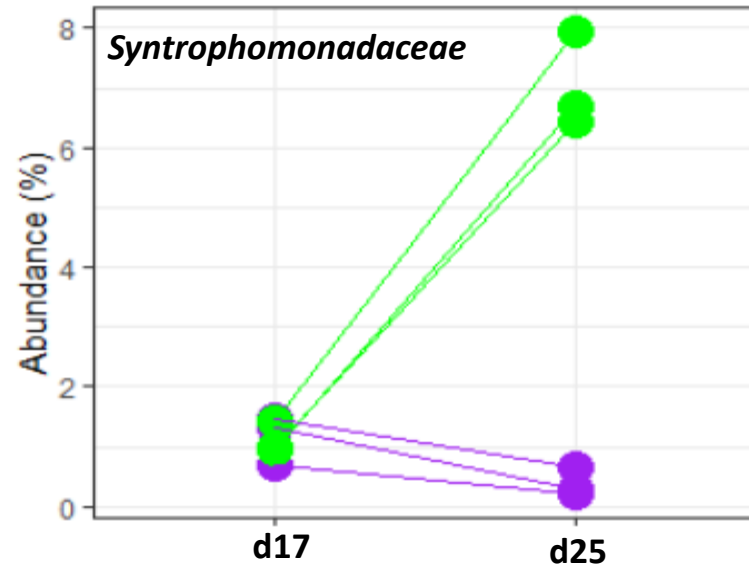
→ 34 matches for the 430 reliable viral contigs

Including 16 matches for the 127 differentially abundant viral contigs

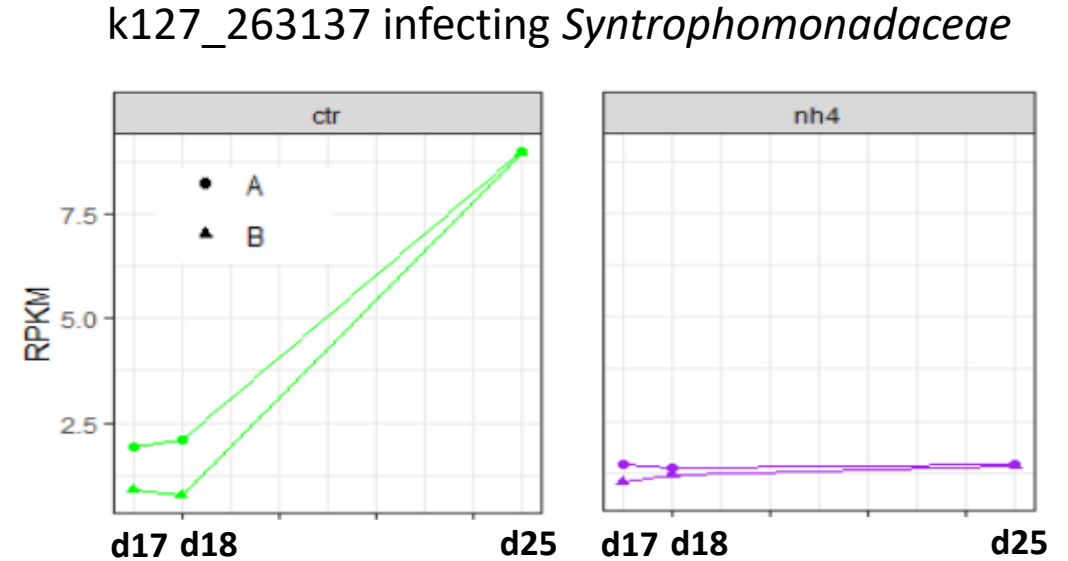
- *Clostridiaceae* is predominant
  - consistent with the 16S analysis
- Two viral contigs predicted to infect *Syntrophomonadaceae*

## ➤ Do host and associated-virus abundances co-evolute ?

### HOST



### VIRAL CONTIG



➔ Important family because it includes **syntrophs**, a functional group at the interface between fermenters and methanogens

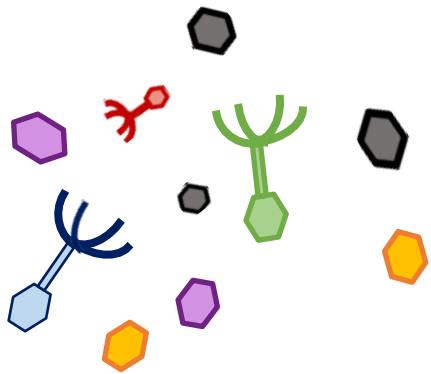
➔ **co-variation** :

When host abundance  = virus abundance 

It supports the following hypothesis :

The composition of viruses changes because the composition of hosts changes... But different cases can co-exist !

## ➤ Conclusions



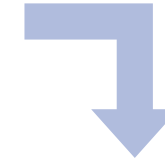
### Viral diversity

- ✓ **127** viral contigs (out of **430**) differentially abundant
- ✓ **16 common** among conditions NaCl, NH<sub>4</sub> and Phenol on day 25

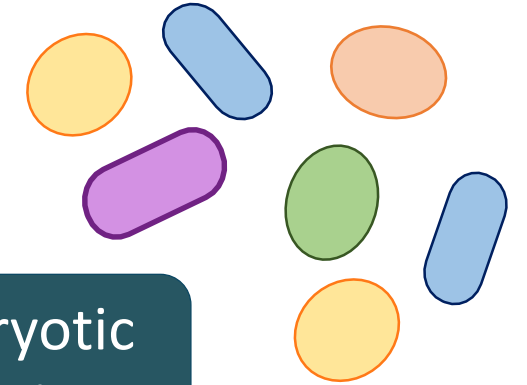


### Global dynamics

- ✓ Significant impact of the tested stresses on methane production



### Prokaryotic diversity



- ✓ *Clostridiaceae* was the most abundant family in all samples
- ✓ **20** distinct genera observed significantly different dynamics

### Virus-Host interaction

There is consistency between :

- production of methane
- differentially abundant *Syntrophomonadaceae*
- differentially abundant associated viral contig

→ Suggesting no provirus activation **in this case...**

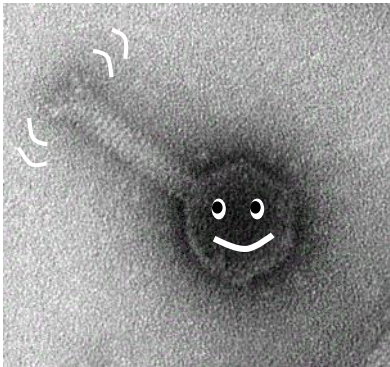
→ Importance of systematic study of contigs of interest

- Assign host, viral family, lifestyle (virulent, temperate)
- See if their variations are correlated to their putative hosts variations
- See if they contain interesting functions

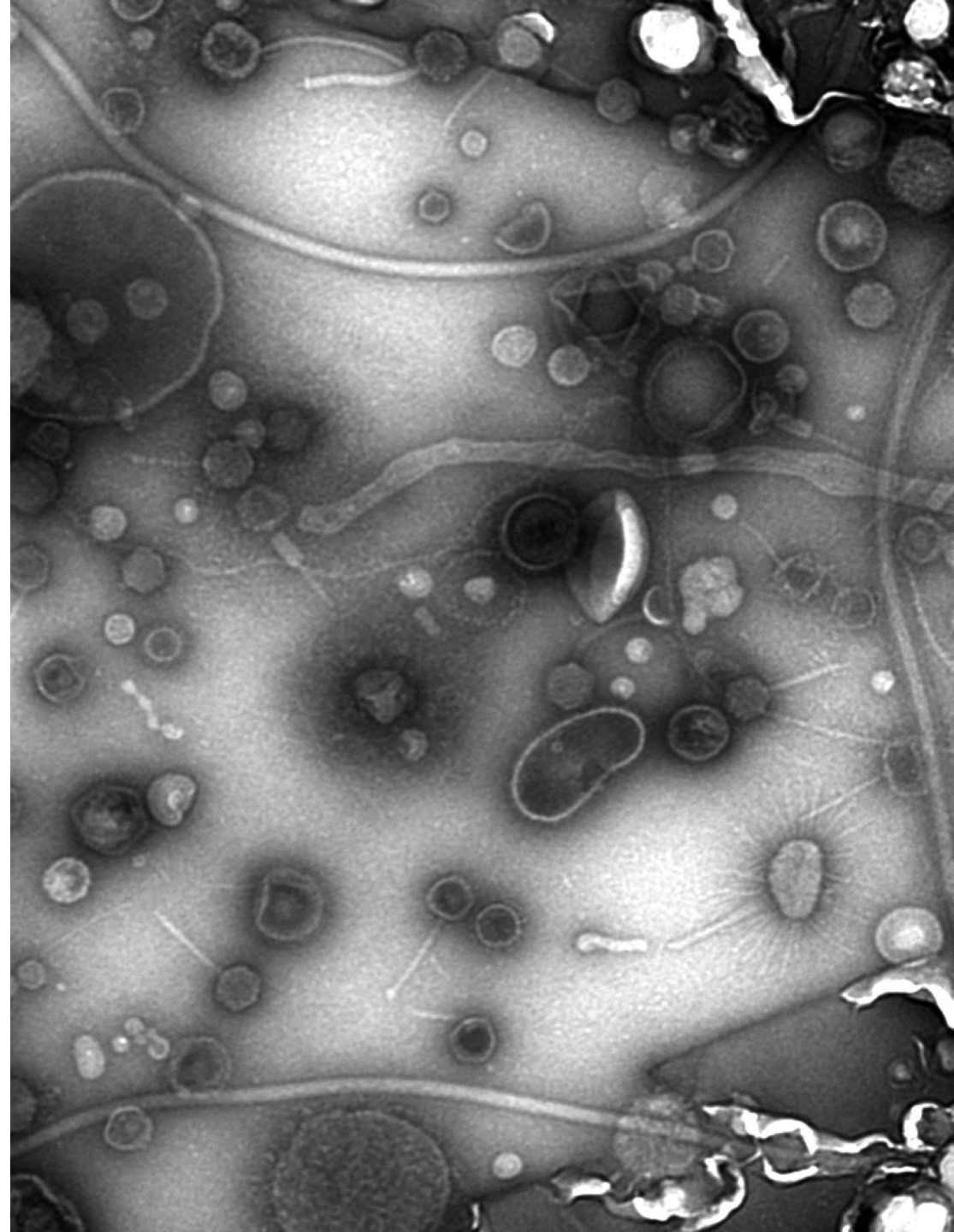


➤ Thanks for listening!

Any question ?



Big thanks to  
my supervisor  
Ariane Bize



Pour cette présentation, je déclare n'avoir aucun conflit d'intérêt



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SFM - Marion Coves

03-10-2022





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SFM - Marion Coves

03-10-2022

# ➤ Anaerobic digestion

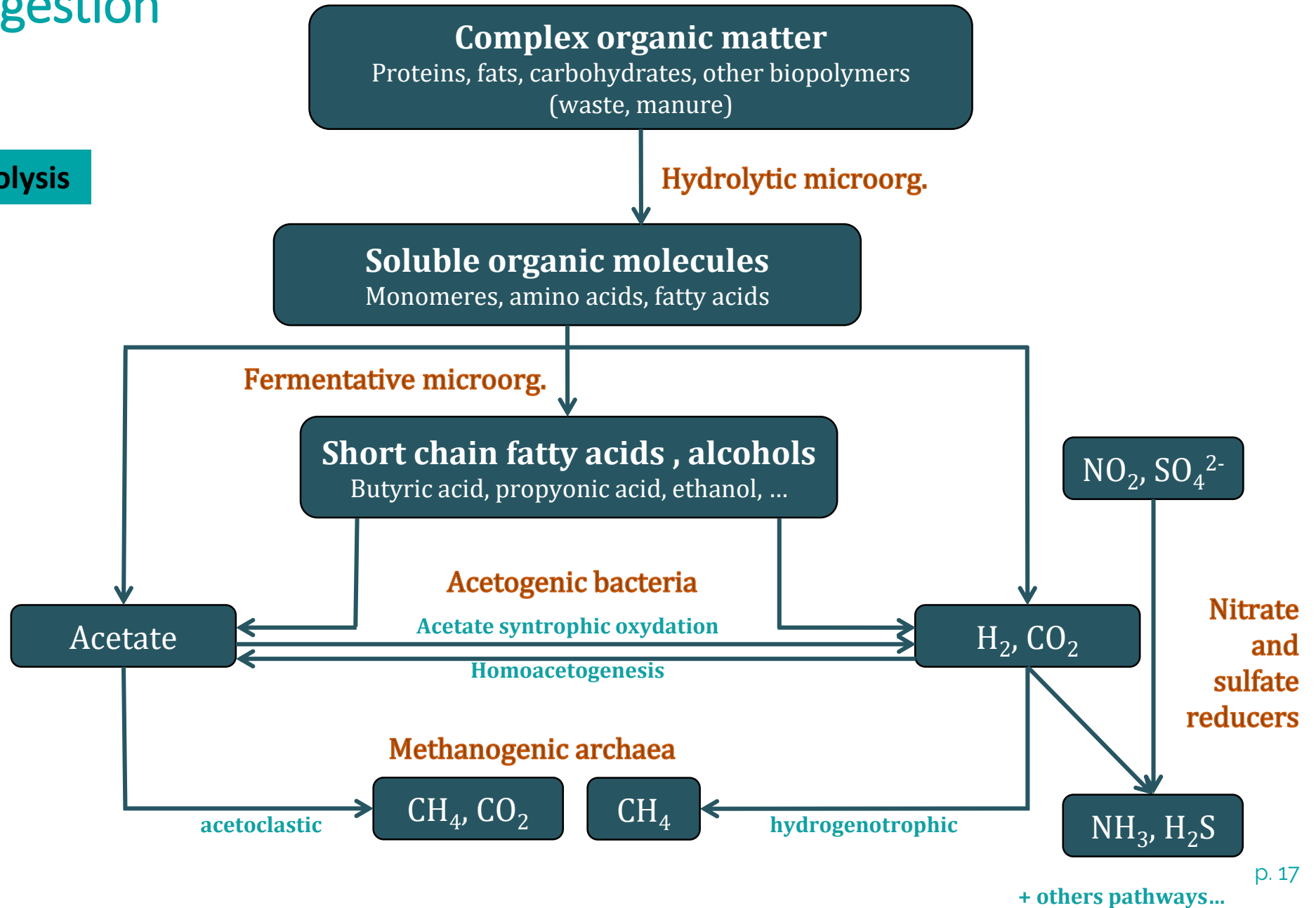
4 steps

Hydrolysis

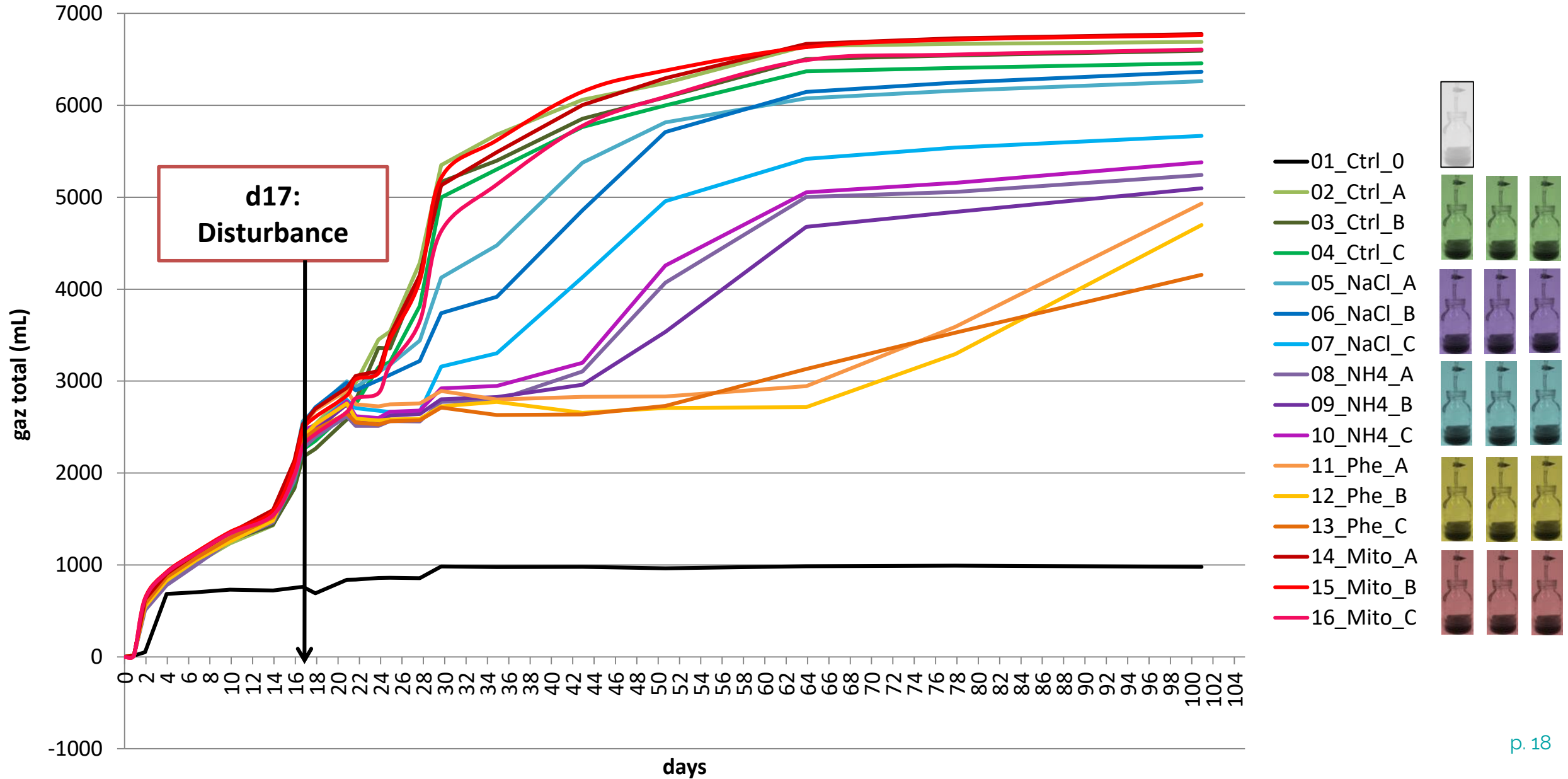
Acidogenesis  
= Fermentation

Acetogenesis

Methanogenesis



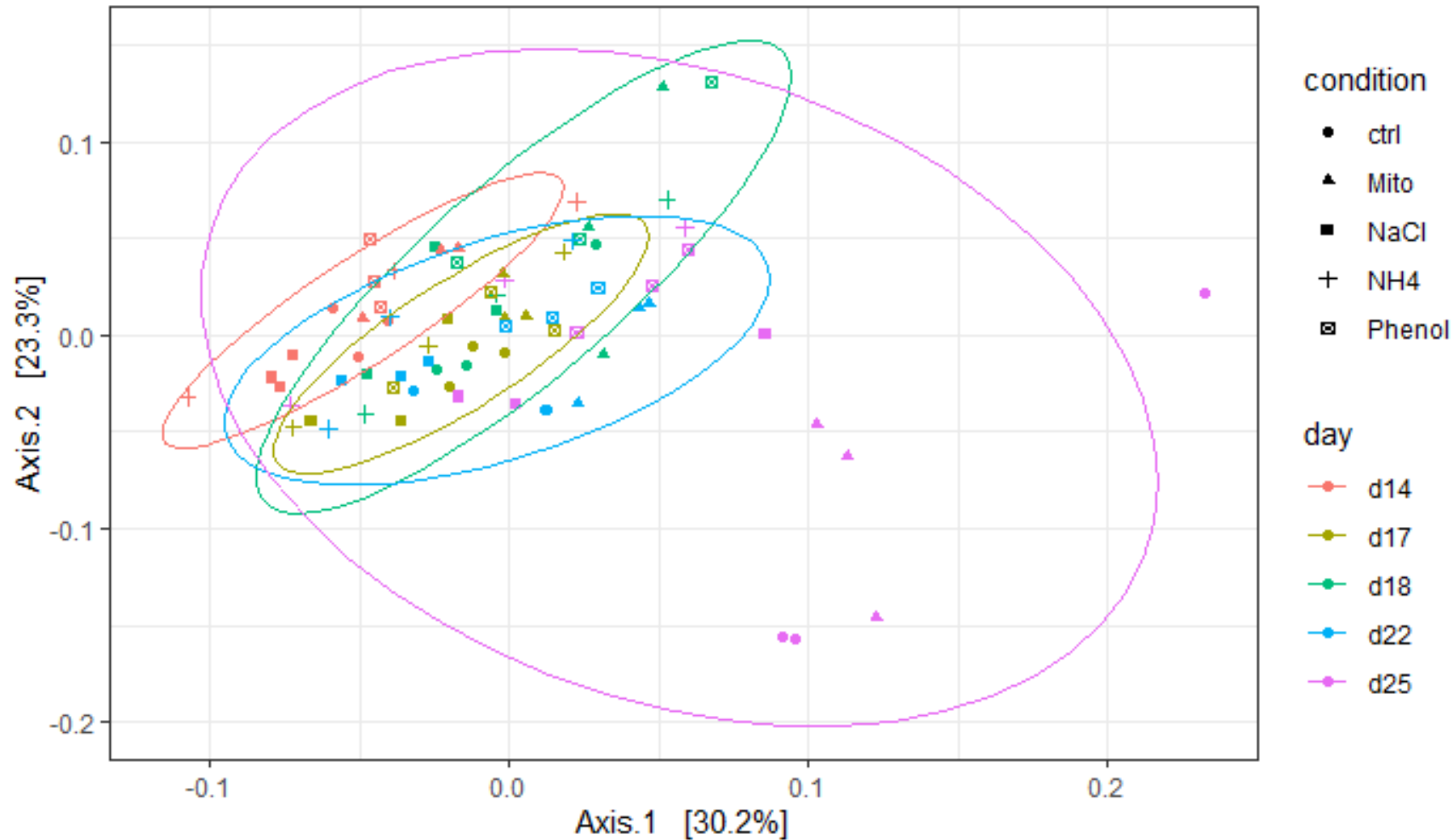
# ➤ Impact on total cumulative gaz production ? YES



# ➤ Impact on microbial community composition at the genus level?

Beta diversity PCoA

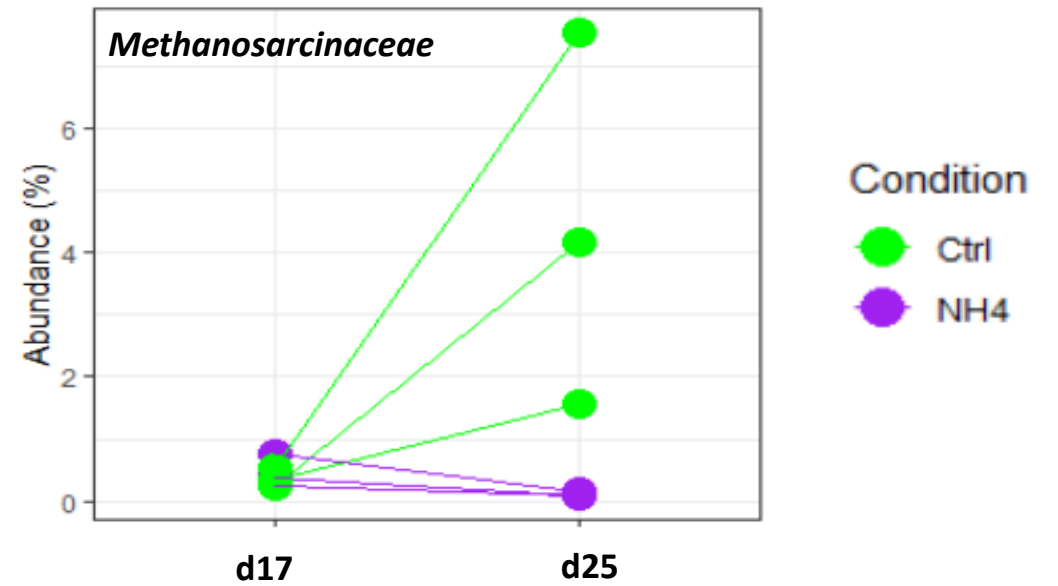
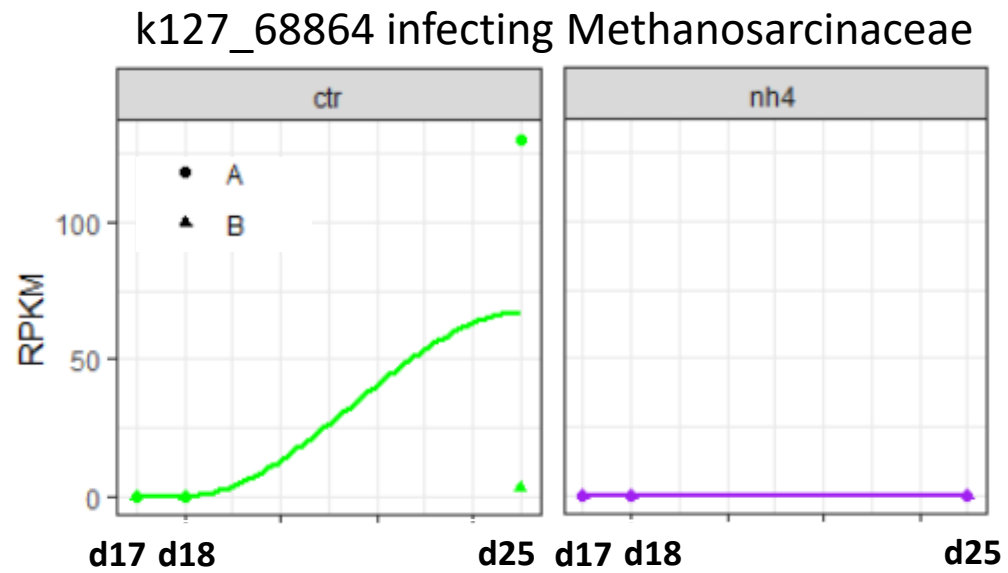
Samples ordination graphic



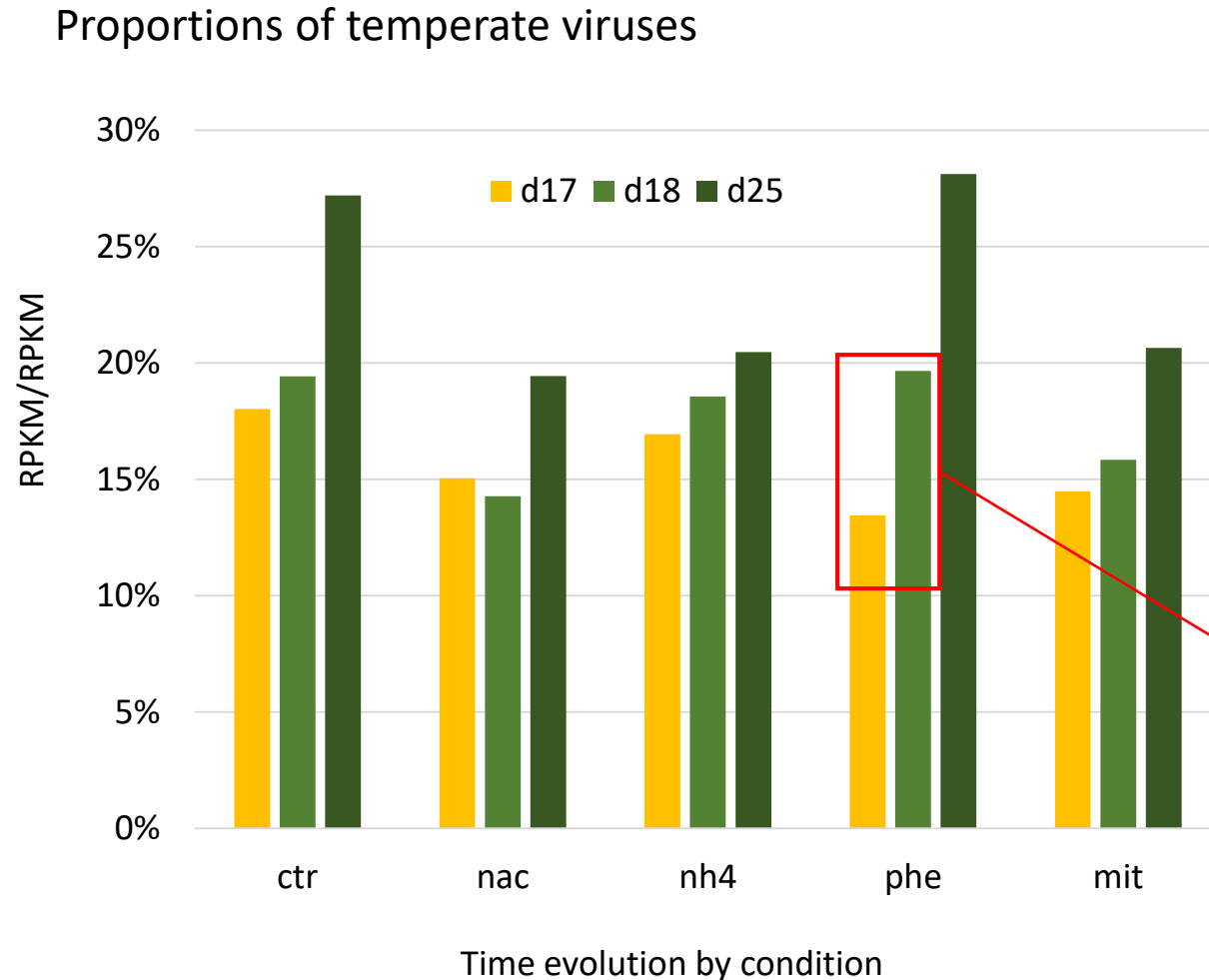
Variation particularly visible on day 25 when looking at the genus level



## ➤ Abundance evolution of methanosarcinaceae



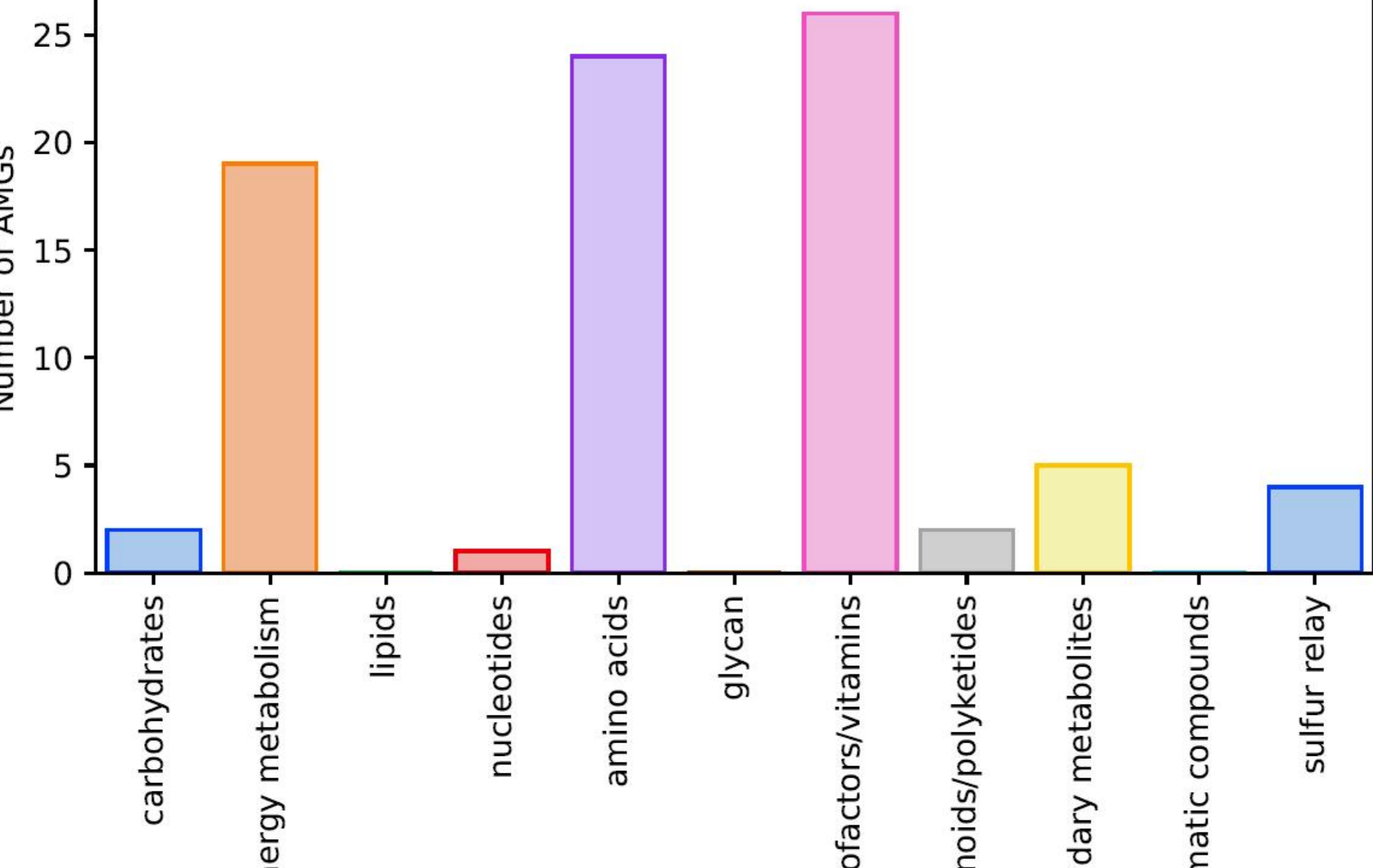
## ➤ What about temperate viruses ?



### Time-bomb hypothesis

Stress could increase the proportion of particles from temperate viruses, by activating the lytic cycle of proviruses.

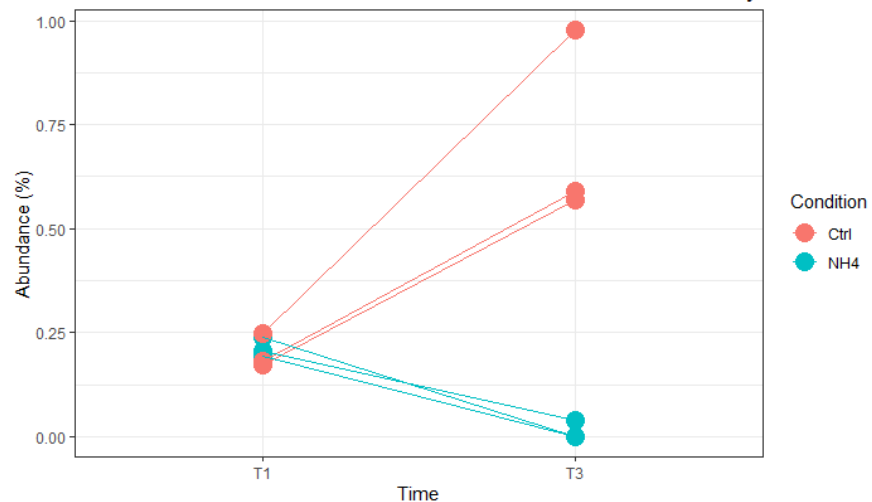
➔ Looking further into the induction of prophages in phenol disturbance experiment could be interesting



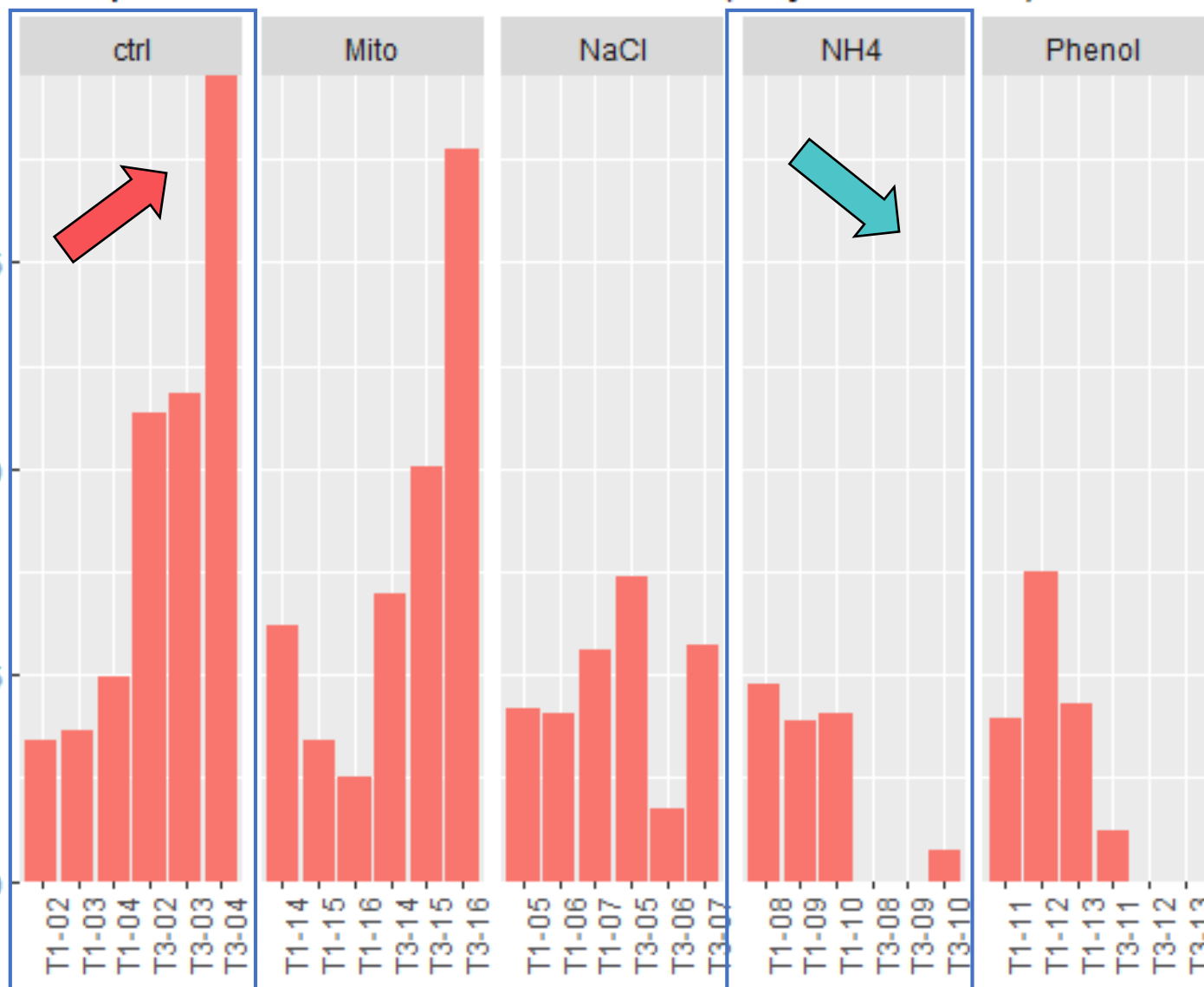
# ➤ Methanoculleus

Log2FoldChange : -6.817845

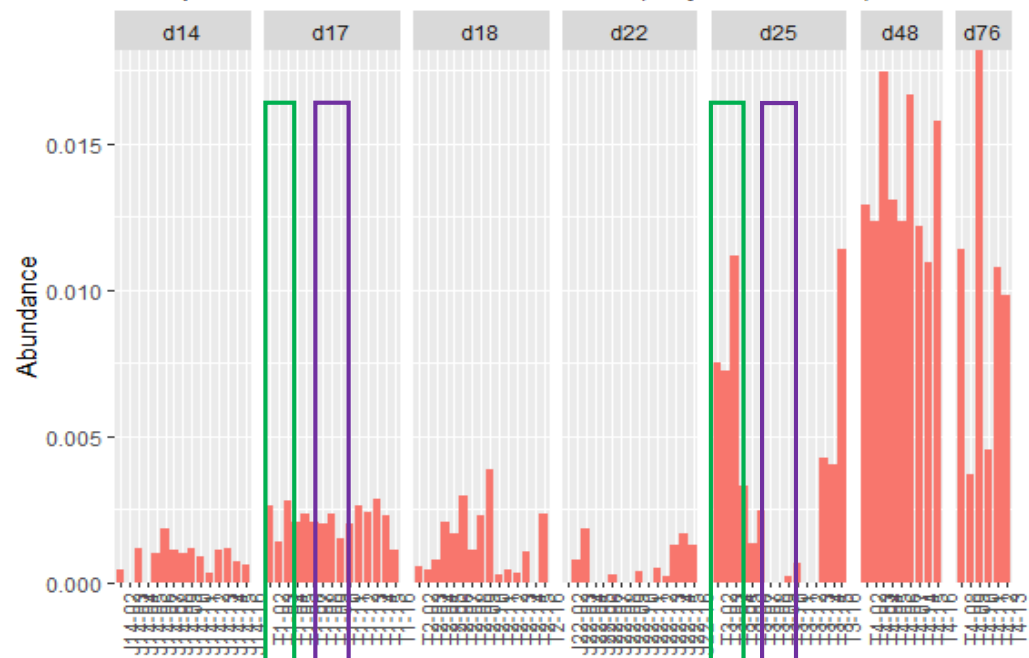
Methanoculleus abundance between NH4 and Ctrl conditions at day 17 and 25



Composition within Methanoculleus (Phylum 1 to 10)



Composition within Methanoculleus (Phylum 1 to 10)

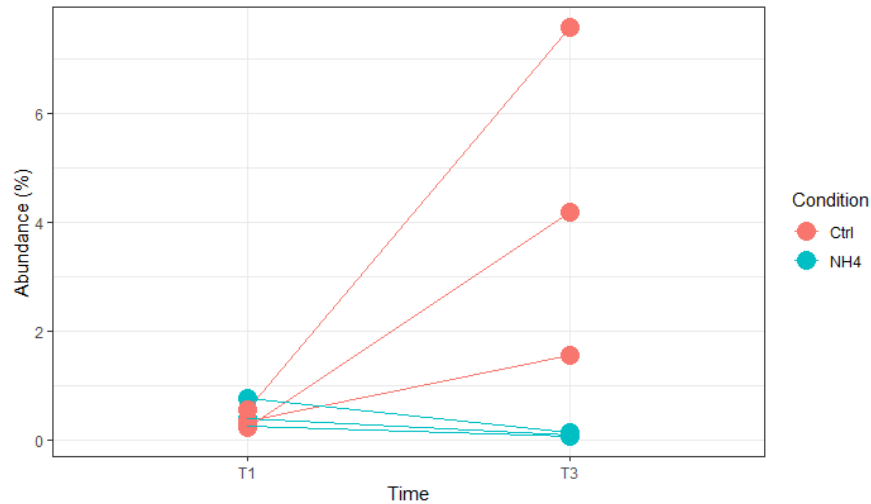




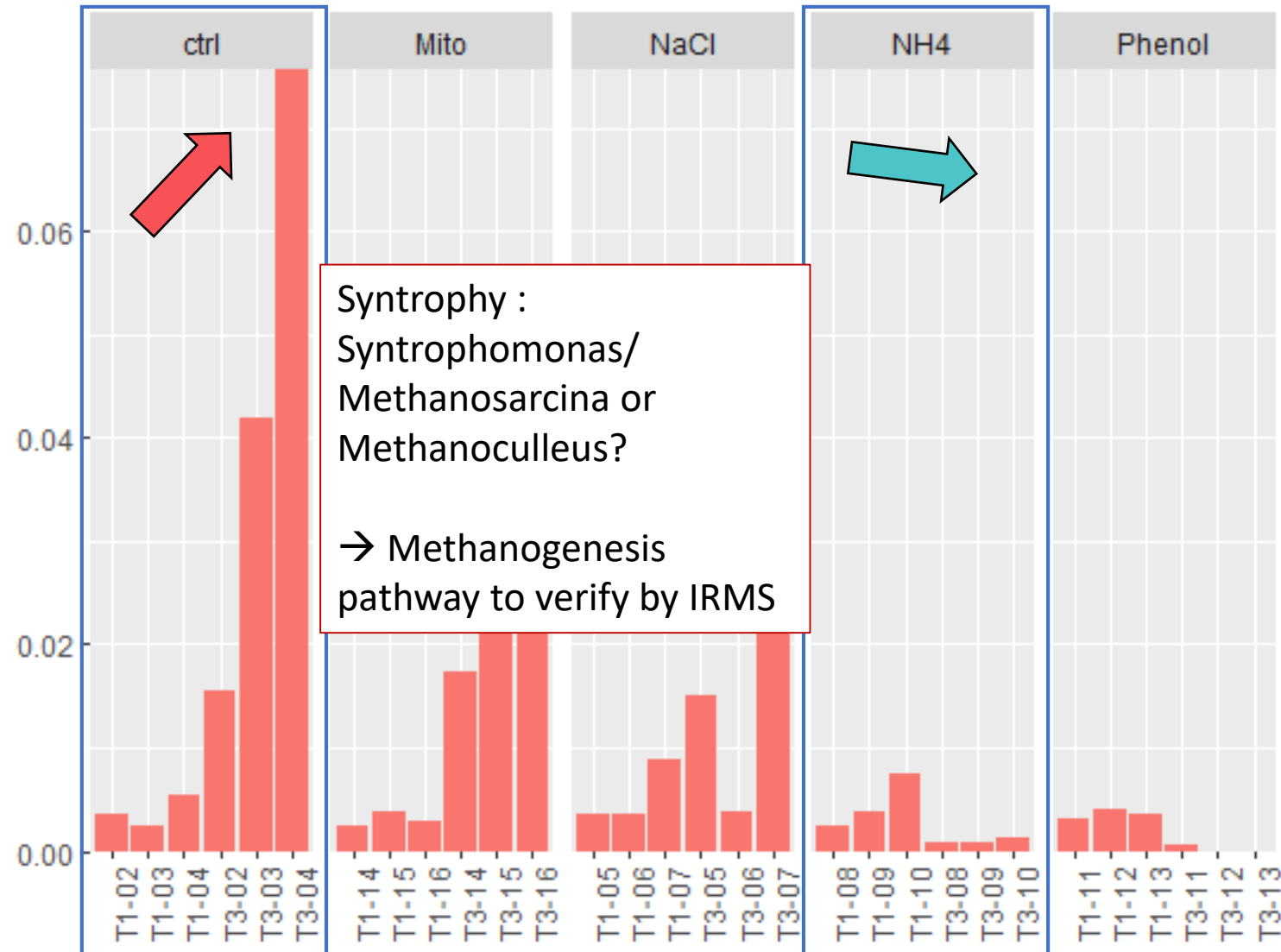
# ➤ Methanosarcina

Log2FoldChange : -5.332194

Methanosarcina abundance between NH4 and Ctrl conditions at day 17 and 25



Composition within Methanosarcina (Phylum 1 to 10)



Composition within Methanosarcina (Phylum 1 to 10)

