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

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

Marion Coves, Laurent Mazéas, Olivier Chapleur,
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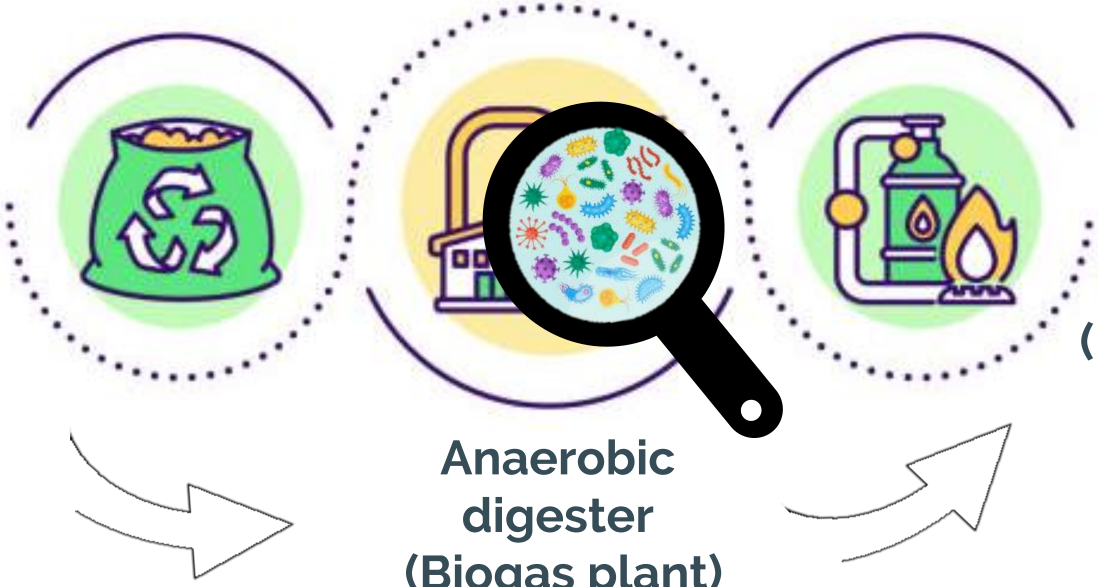
ANR-17-CE05-0011

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



Biowaste

Anaerobic digester (Biogas plant)

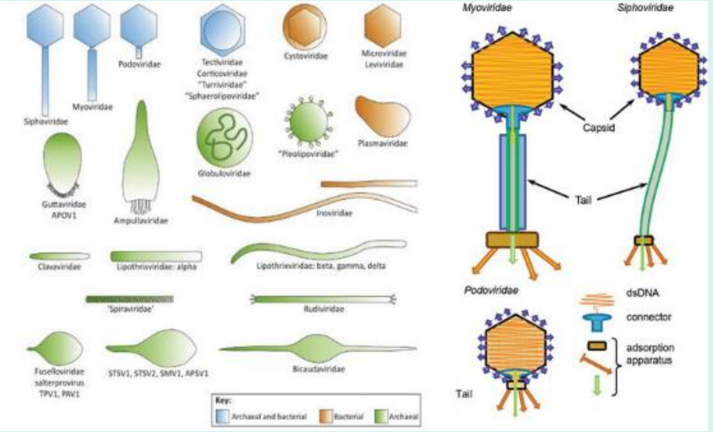
Biogas (Methane)

=

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

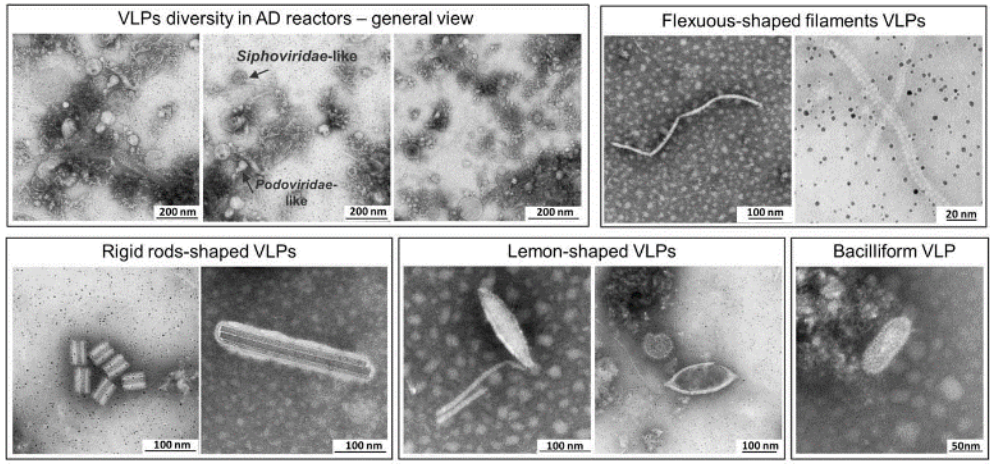
Statement: **Inhibition can impact the microbial community directly and indirectly.**

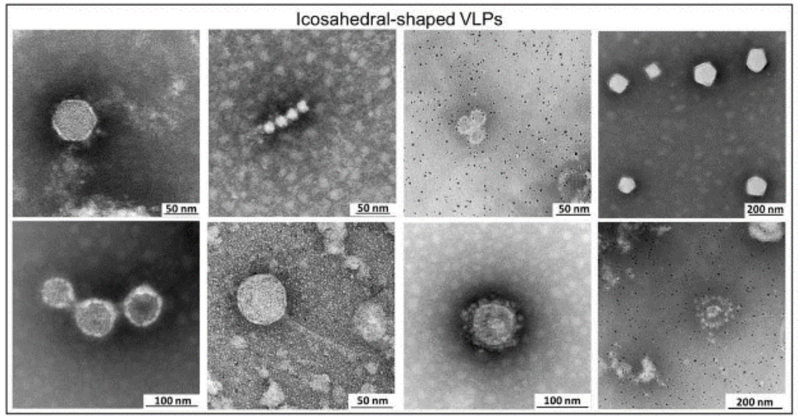
Question: **Is the viral community one of the indirect factors ?**

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



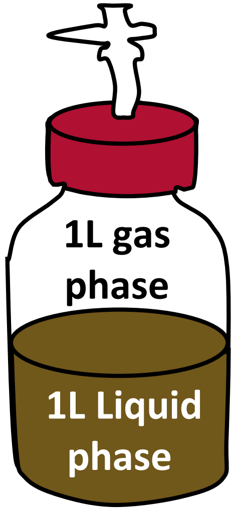


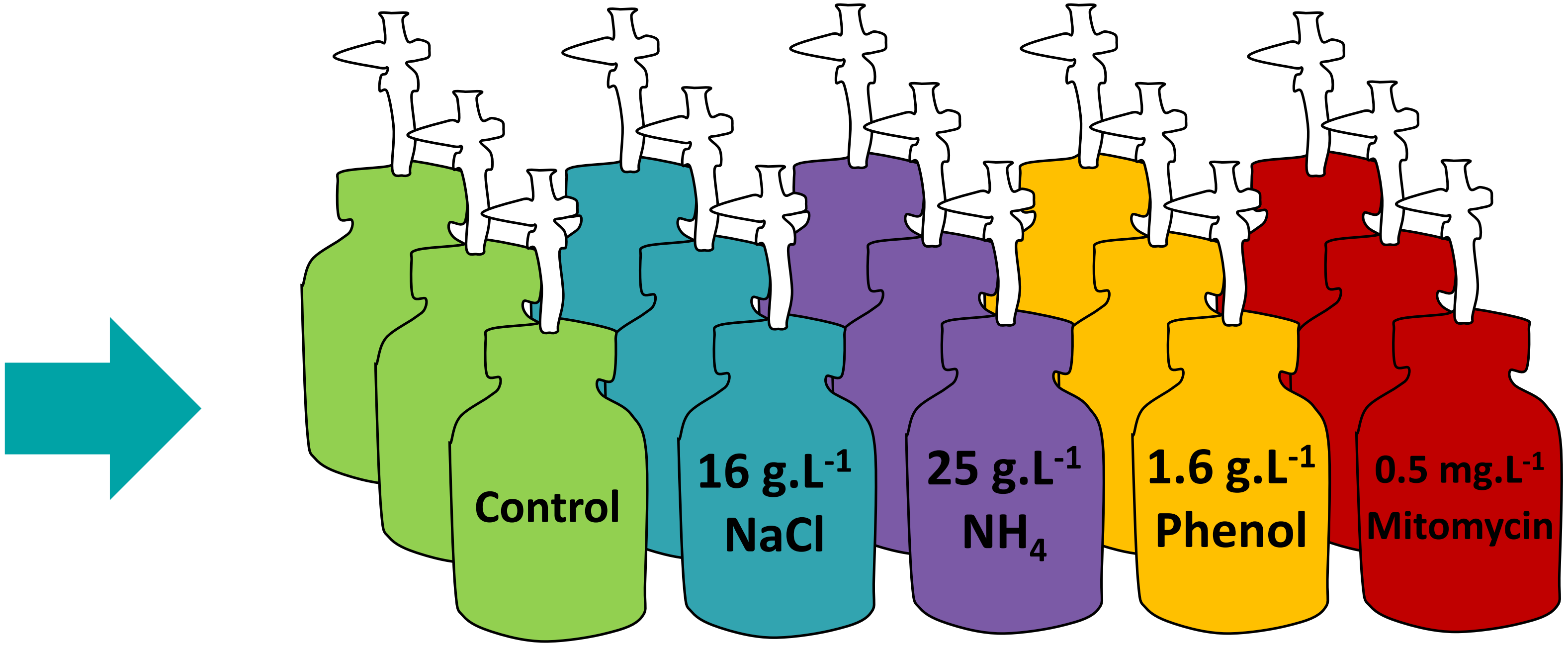
Calusinska *et al,* 2016 Environmental microbiology

Experimental design

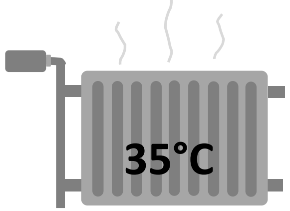
Initial content

- Nitrogen (N_2)
- Biowaste
- Mineral medium
- Inoculum from a lab AD pilot





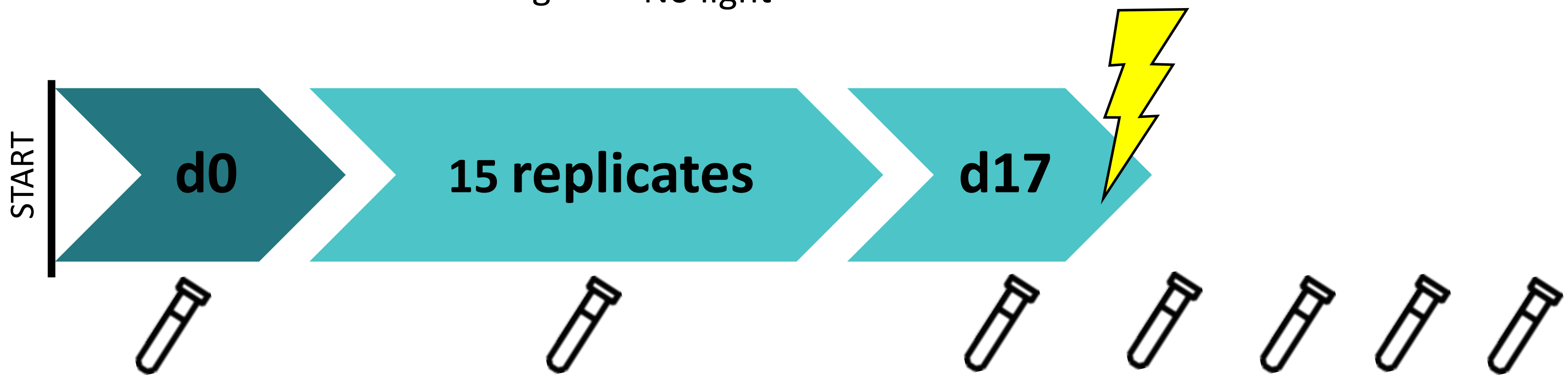
3 identical replicates for each condition



No stirring

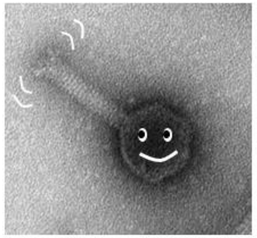
No light

Addition of a disturbance



Liquid and gas sampling

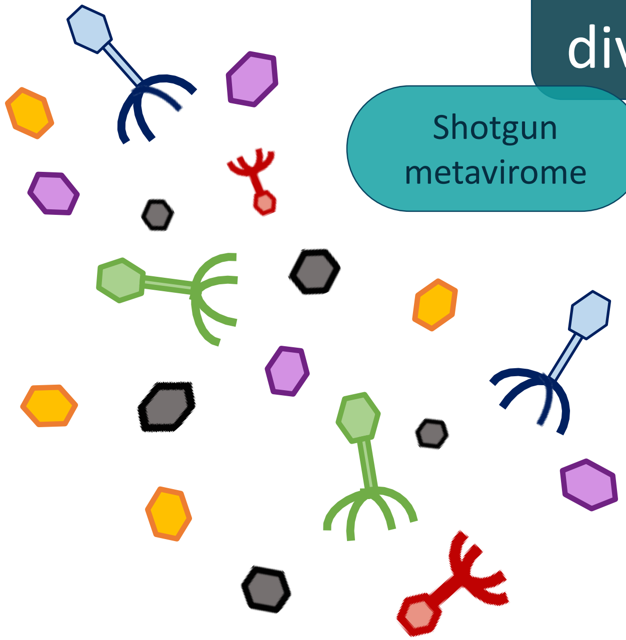
Experimental design


(Non-contractual picture)

Electron microscopy

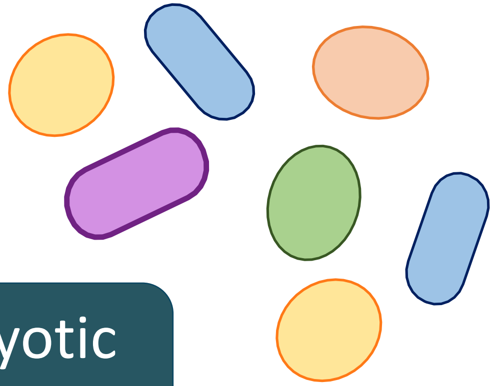
Viral diversity

Shotgun metavirome



Global dynamics

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



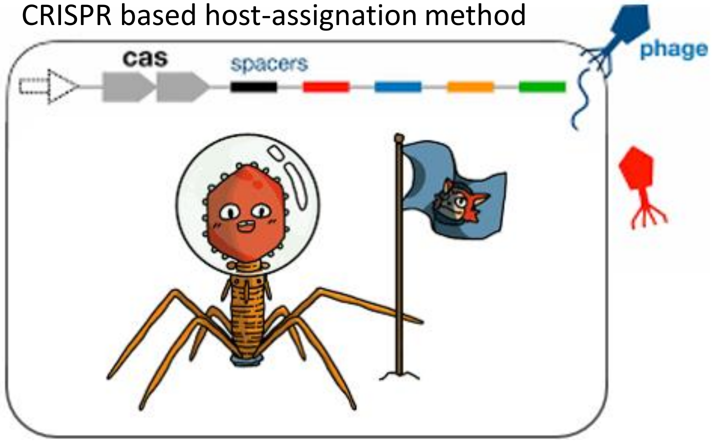
Prokaryotic diversity

ADNr 16S Metabarcoding

Virus-Host interaction and ecosystem functioning

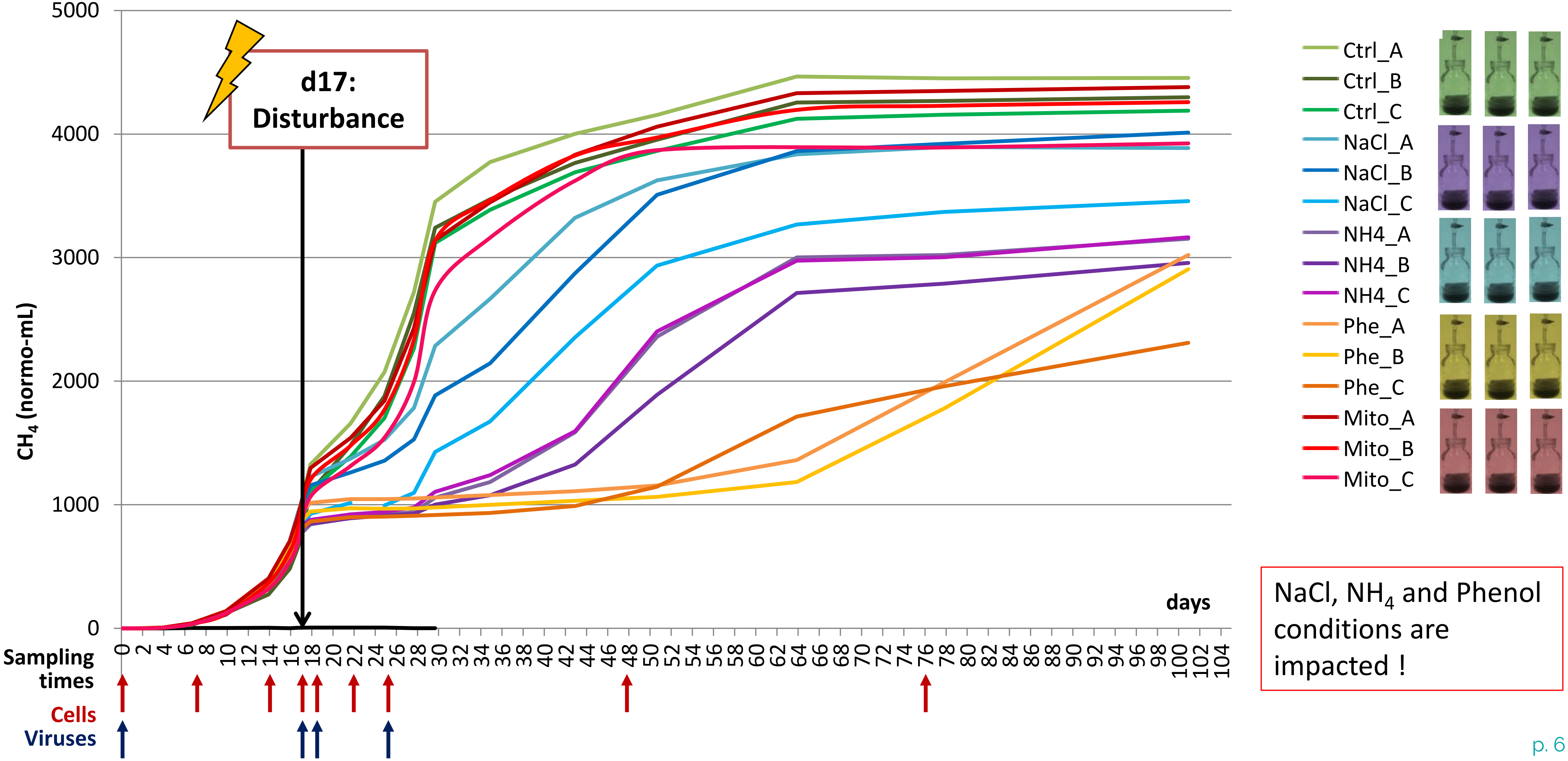
Bioinformatics approaches

CRISPR based host-assignation method



Ruoshi Zhang, *et al*. 2021, SpacePHARER

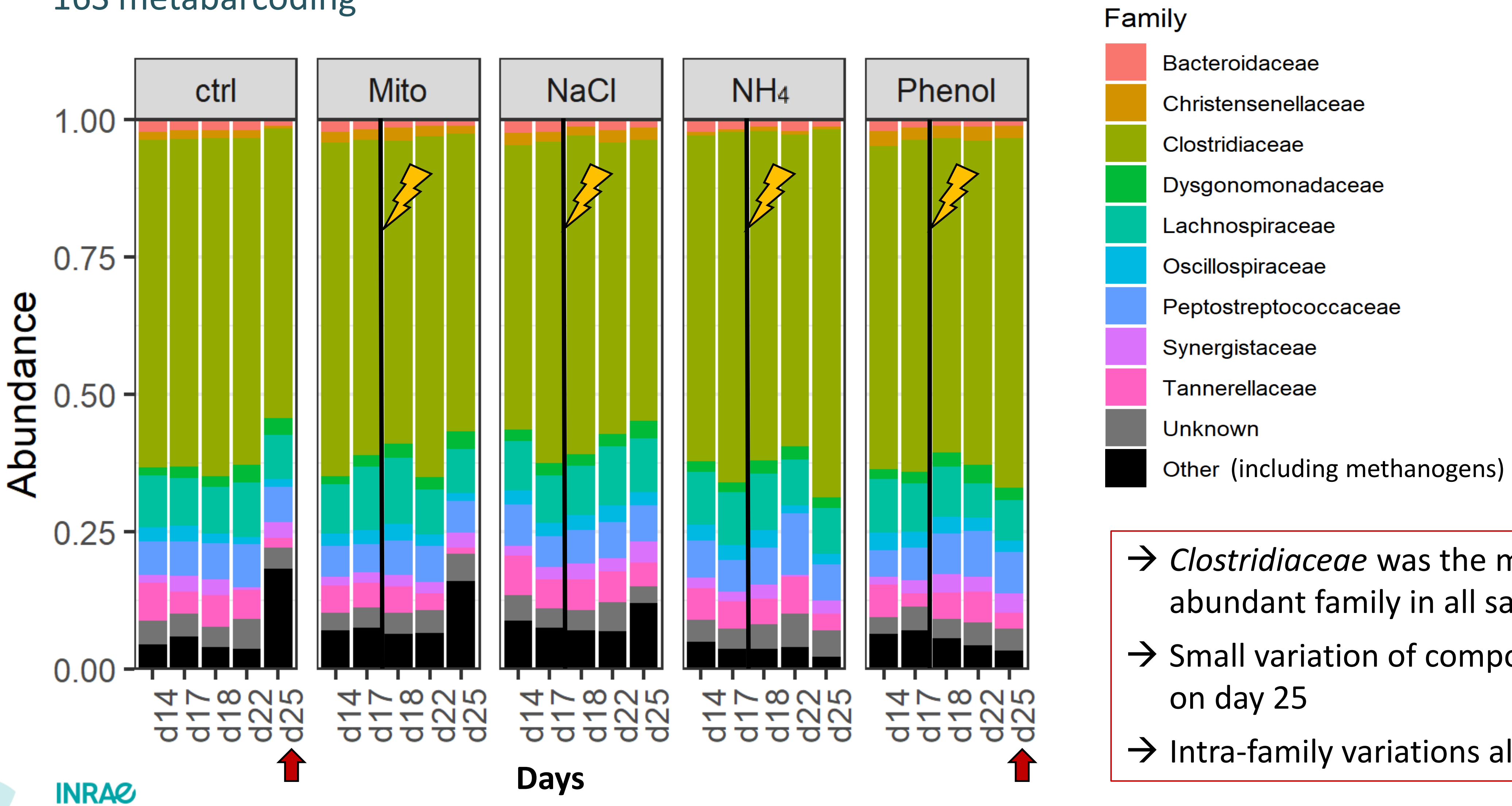
Impact on CH_4 cumulative production?



NaCl, NH_4 and Phenol conditions are impacted !

Impact on microbial community composition at the family level?

16S metabarcoding



→ *Clostridiaceae* was the most abundant family in all samples

→ Small variation of composition on day 25

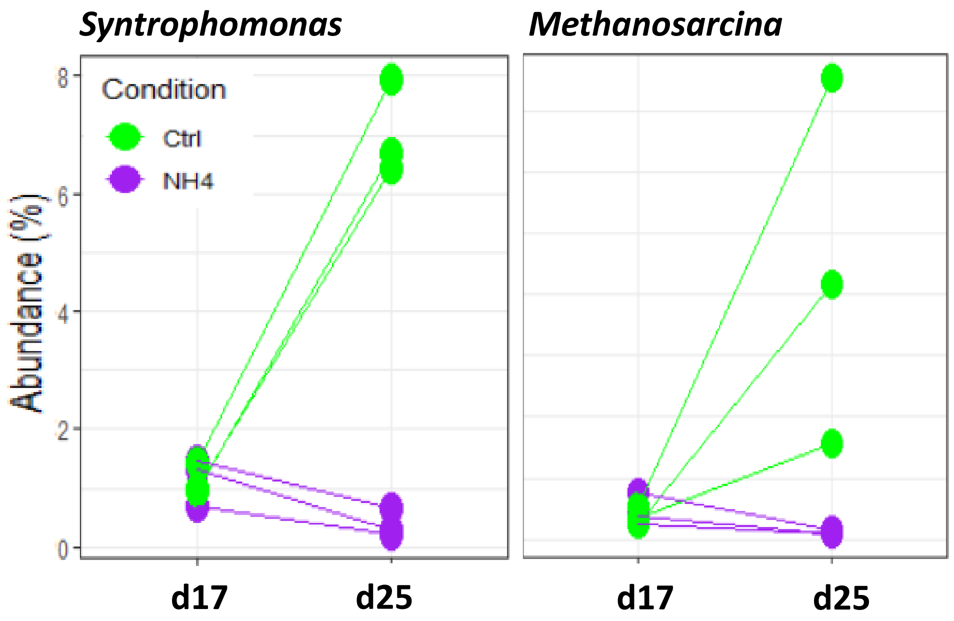
→ Intra-family variations also occur

How many genera are affected by the disturbances?

Differential abundance analysis

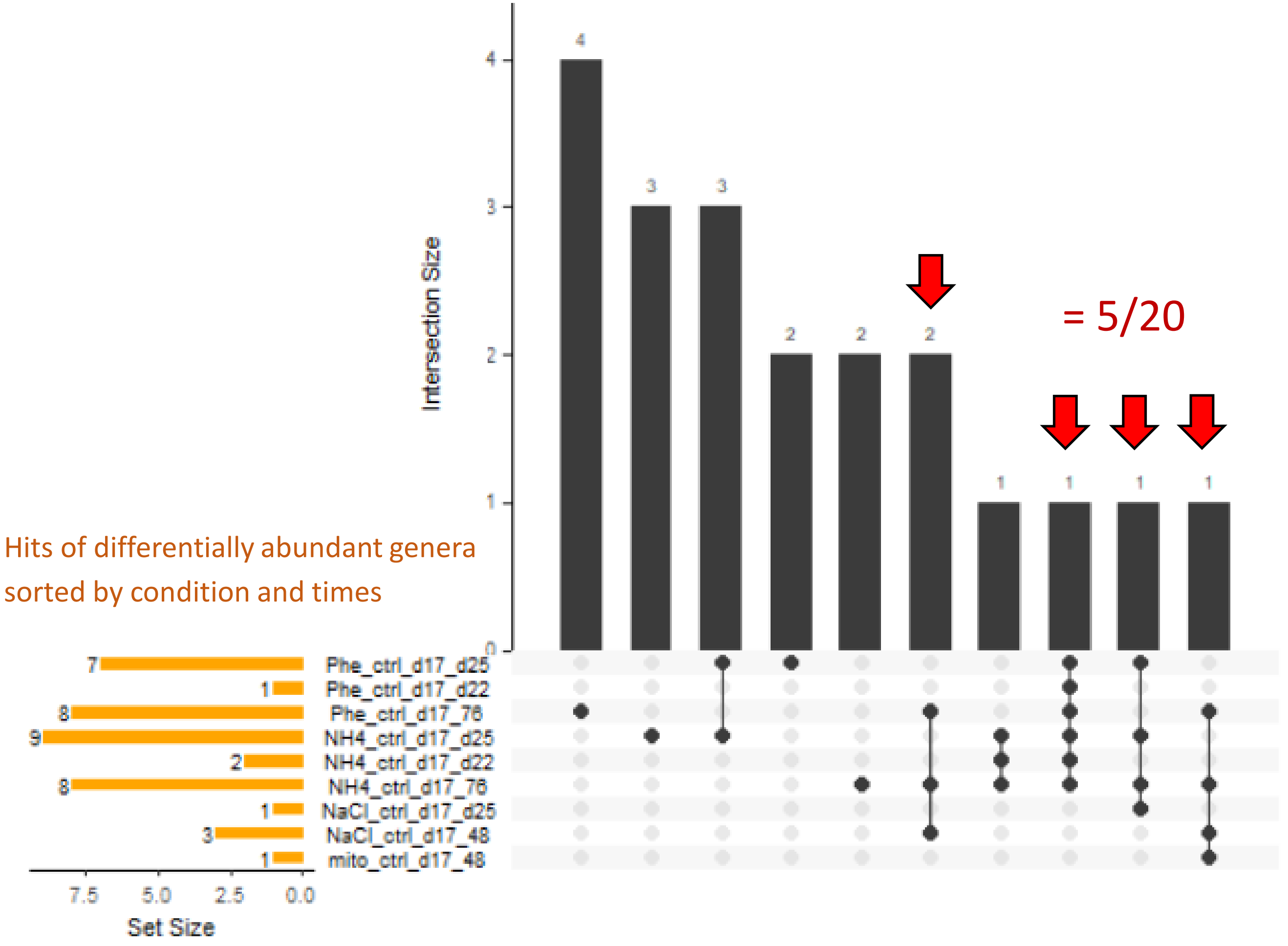
Differentially abundant genera between :

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



Syntrophomonas

Methanosarcina



Hits of differentially abundant genera sorted by condition and times

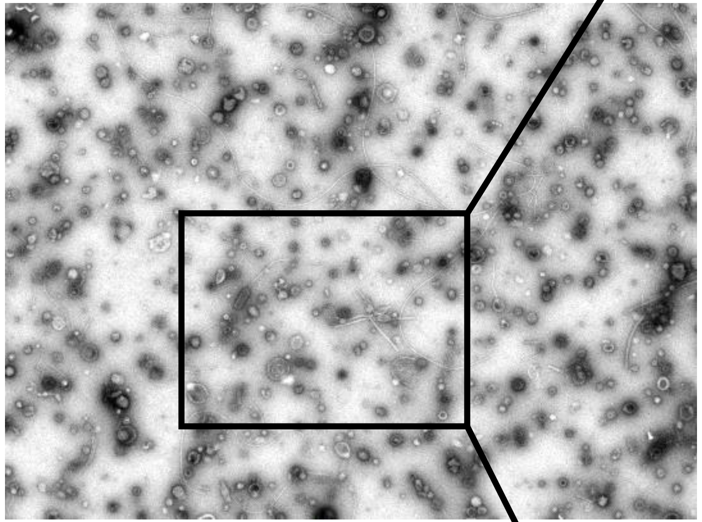
→ *Syntrophomonas* and *Methanosarcina* genera abundance evolutions are consistent with biogas production inhibition

→ 20 distinct genera observed significantly different dynamics

→ 5 are common to the three most inhibited conditions with NaCl, NH_4, Phenol

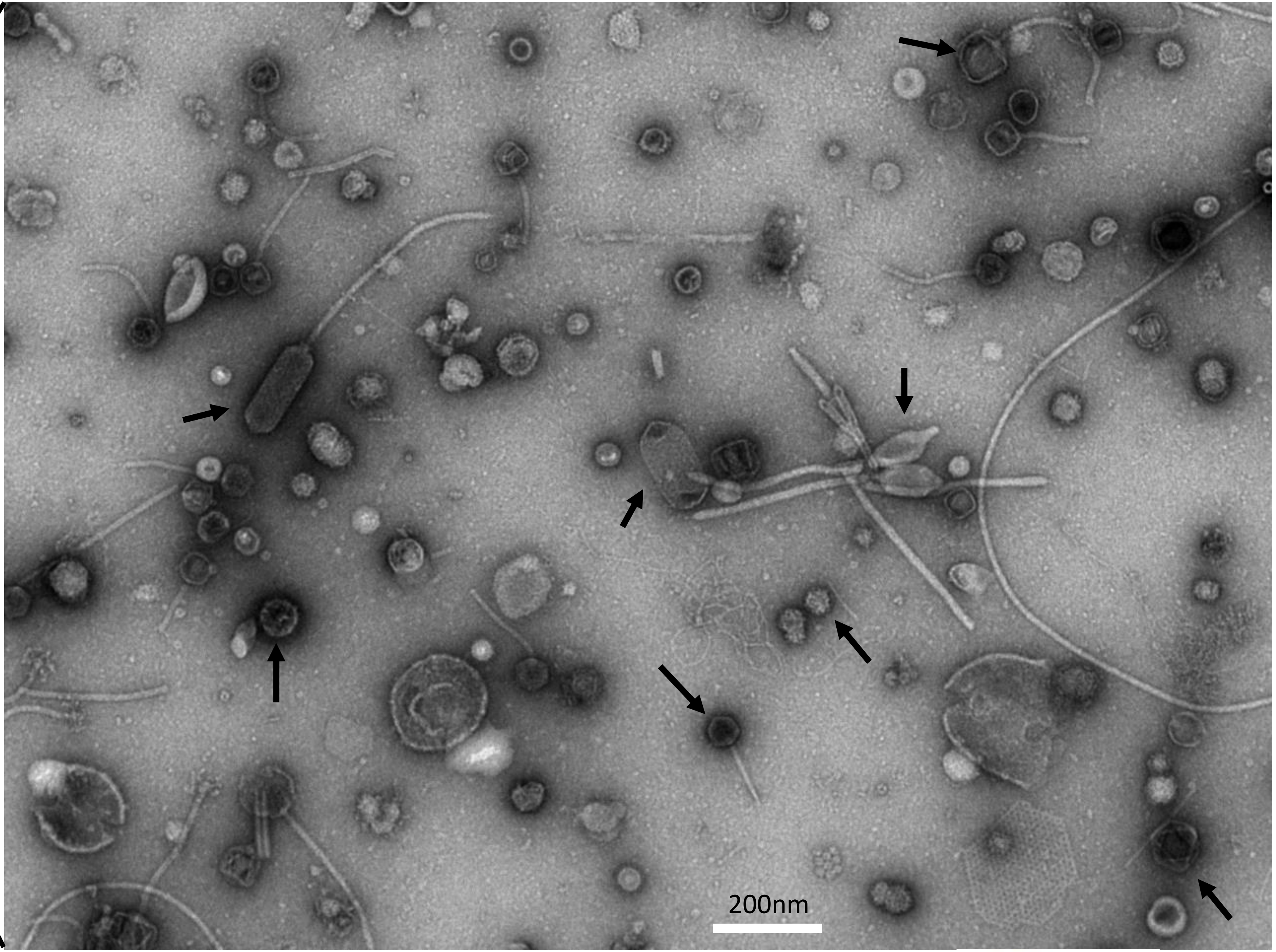
Viruses, Are you here?

Electron microscopy



- Viral particles isolated by filtration (0.22µm)
- 690ng per sample of DNA extracted in average

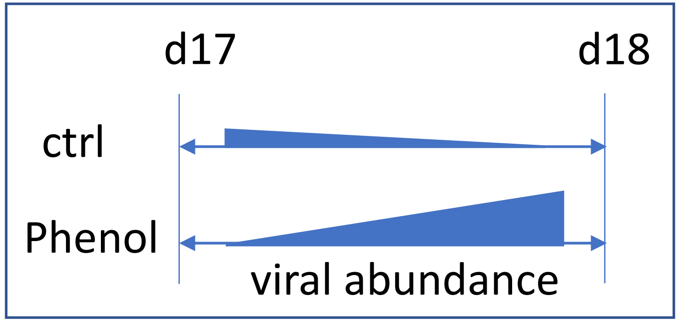
→ Viral particles are numerous and diverse in shapes and sizes



How many viruses are affected by the disturbances ?

Differential abundance analysis

example

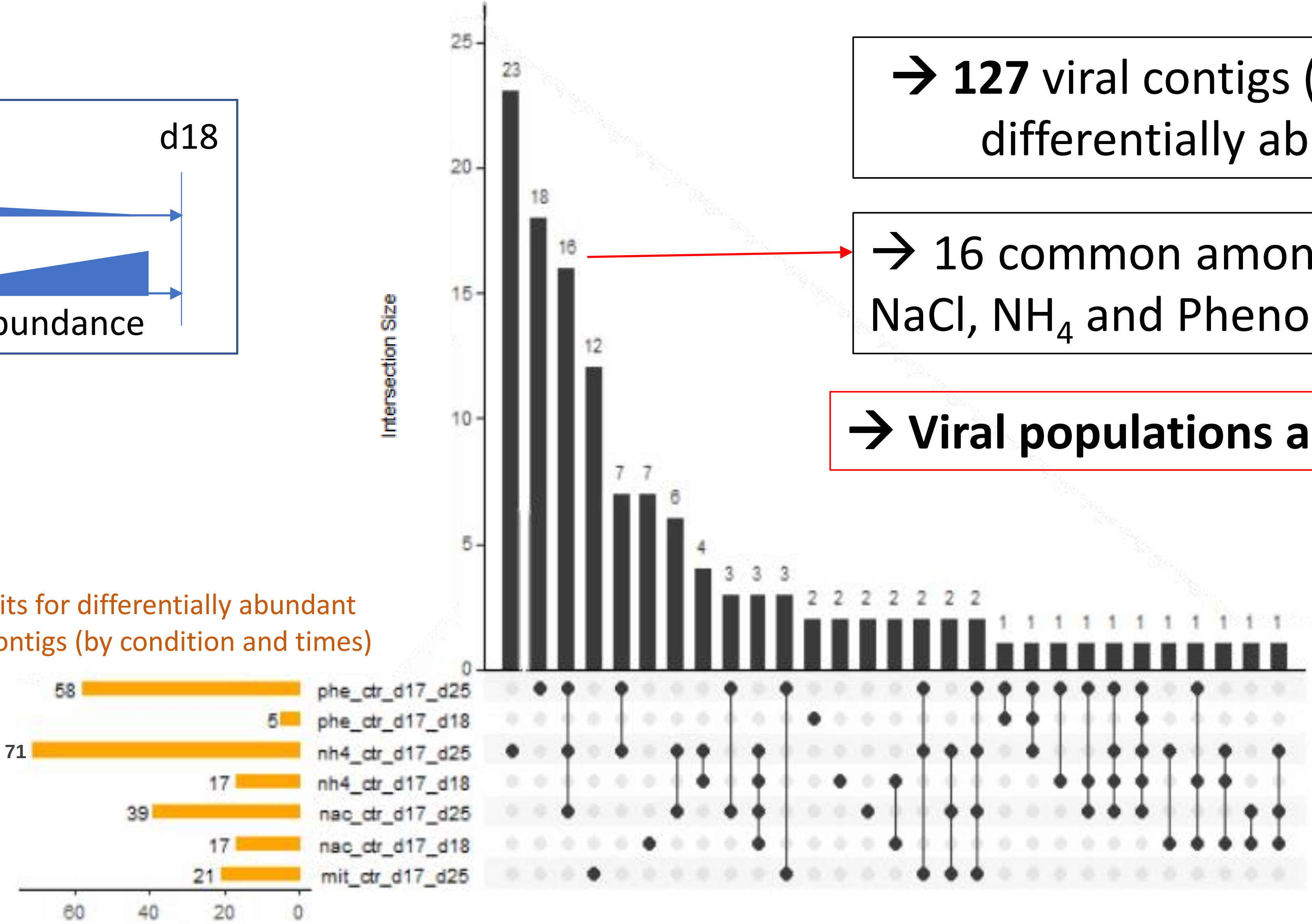


→ **127** viral contigs (out of **430**) differentially abundant

→ 16 common among conditions NaCl, NH_4 and Phenol on day 25

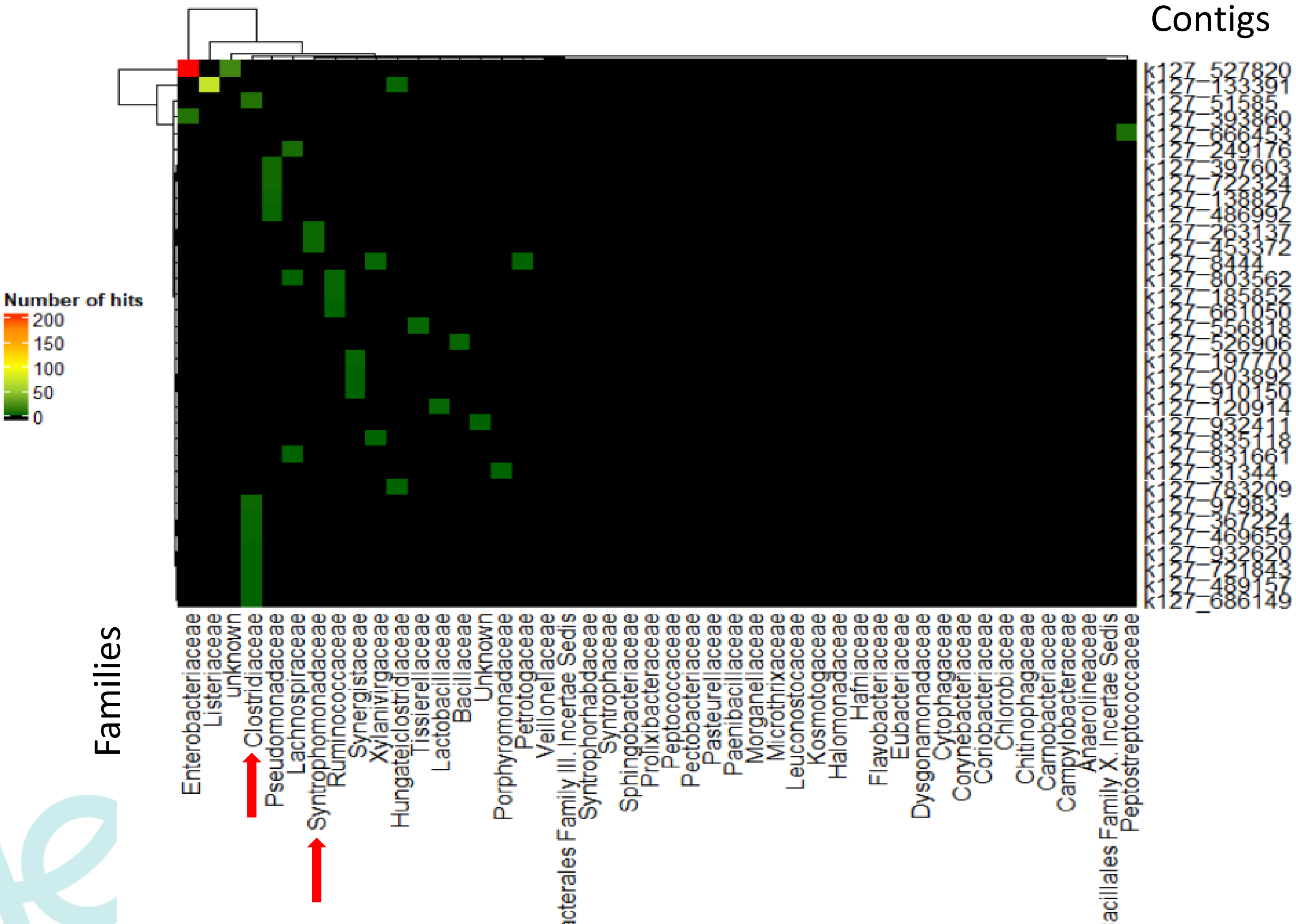
→ Viral populations are impacted

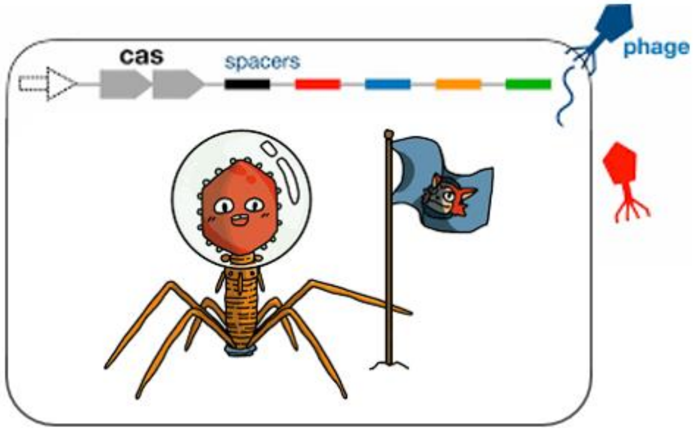
Hits for differentially abundant contigs (by condition and times)



Who are the hosts of these viruses?

CRISPR-spacer based host-assignation method (SpacePharer)





Ruoshi Zhang, *et al*. 2021, SpacePHARER

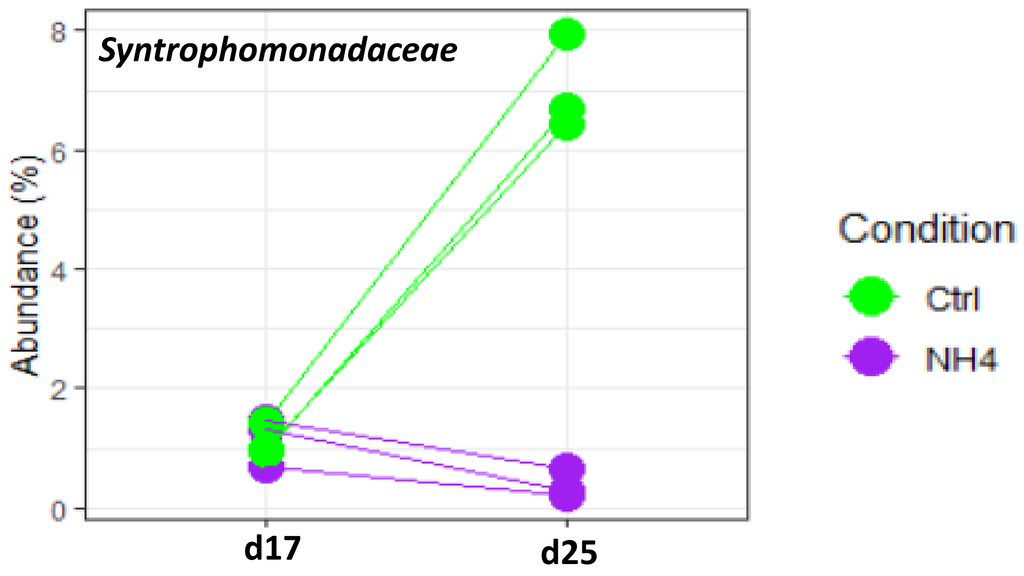
→ 34 matches for the 430 reliable viral contigs

Including 16 matches for the 127 differentially abundant viral contigs

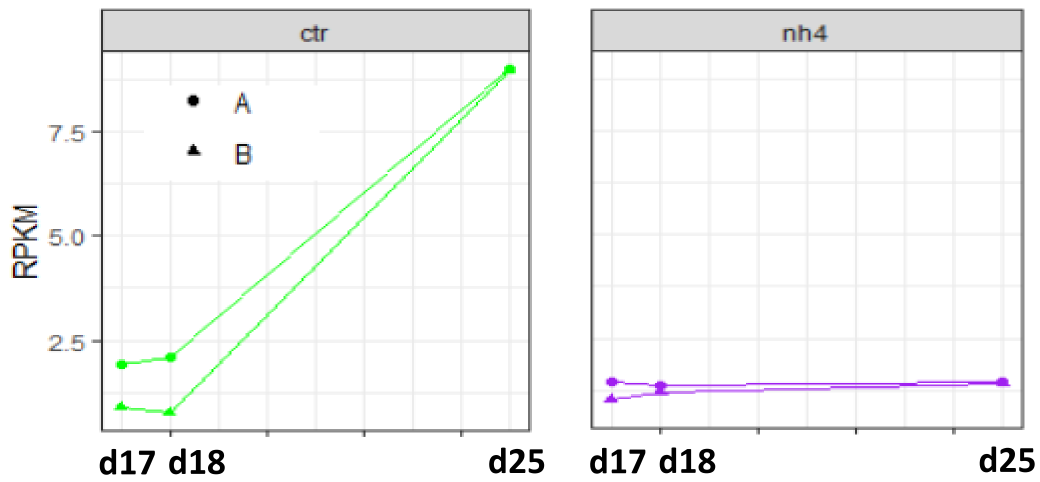
- *Clostridiacaea* is predominant
  →consistent with the 16S analysis
- Two viral contigs predicted to infect *Syntrophomonadacaea*

Do host and associated-virus abundances co-evoluate ?

HOST



VIRAL CONTIG


k127_263137 infecting *Syntrophomonadaceae*

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

→ co-variation :

When host abundance ↗ = virus abundance ↗

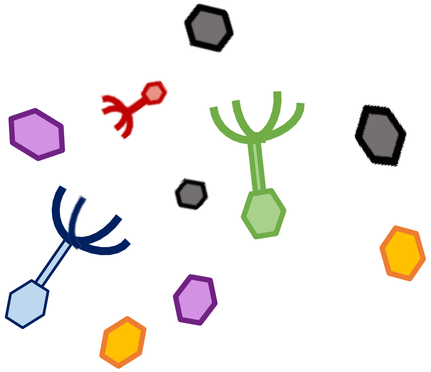
It supports the following hypothesis :

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

Conclusions

Global dynamics

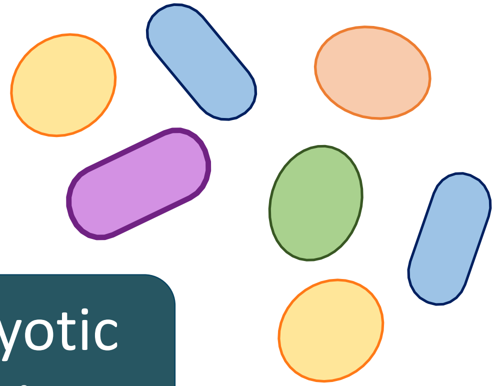
✓ Significant impact of the tested stresses on methane production



Viral diversity

✓ **127** viral contigs (out of **430**) differentially abundant

✓ **16 common** among conditions NaCl, NH_4 and Phenol on day 25



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 *Syntrophomonadacaea*
- differentially abundant associated viral contig

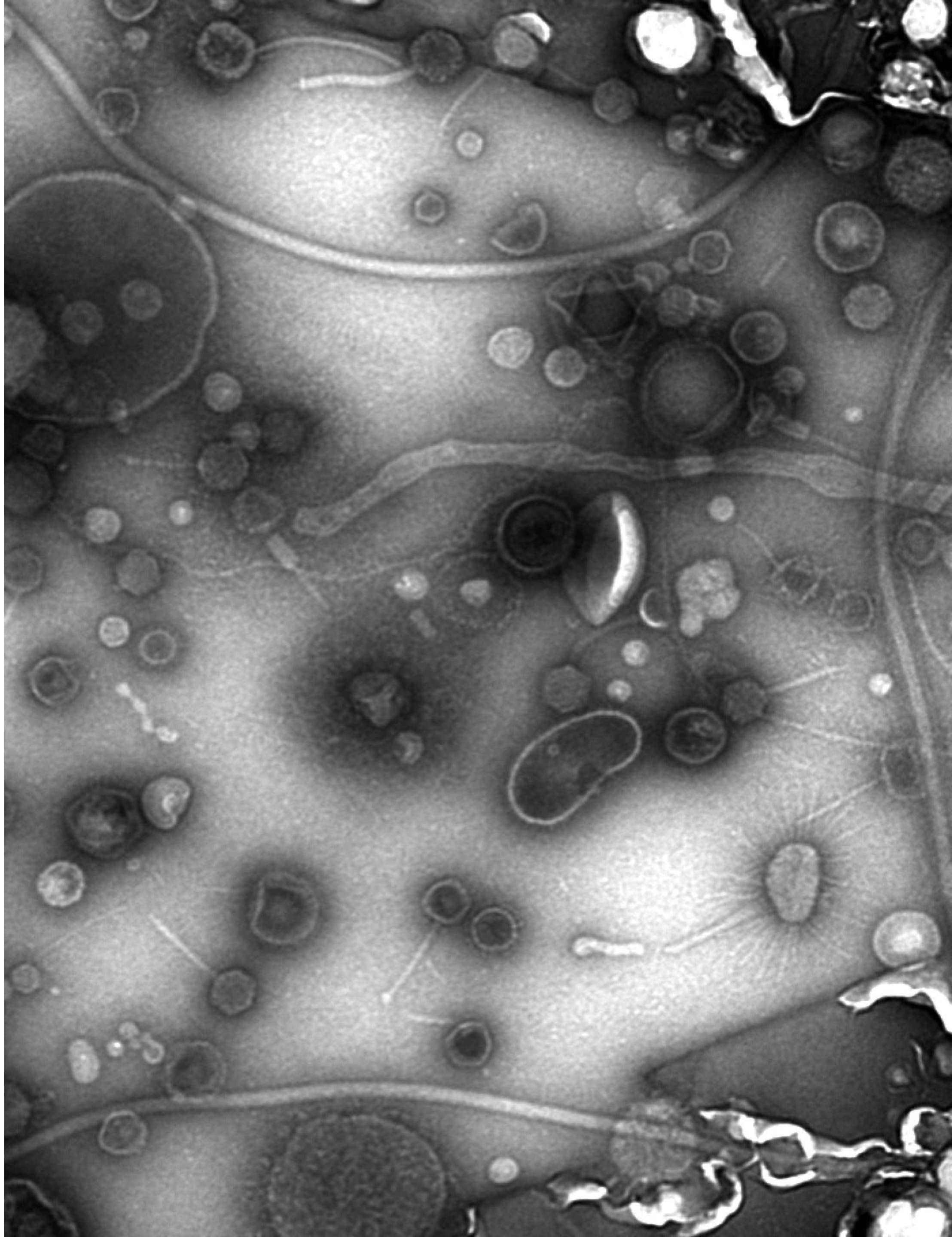
→ Suggesting no provirus activation **in this case**…

→ Importance of systematic study of contigs of interest

❑ Assign host, viral family, lifestyle (virulent, temperate)

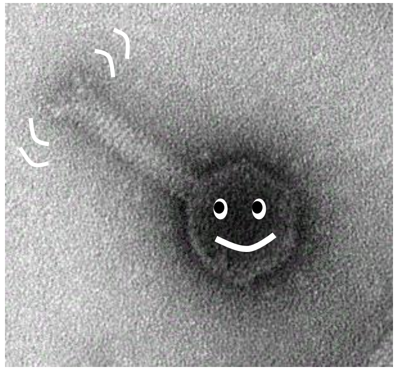
❑ See if their variations are correlated to their putative hosts variations

❑ 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

Anaerobic digestion

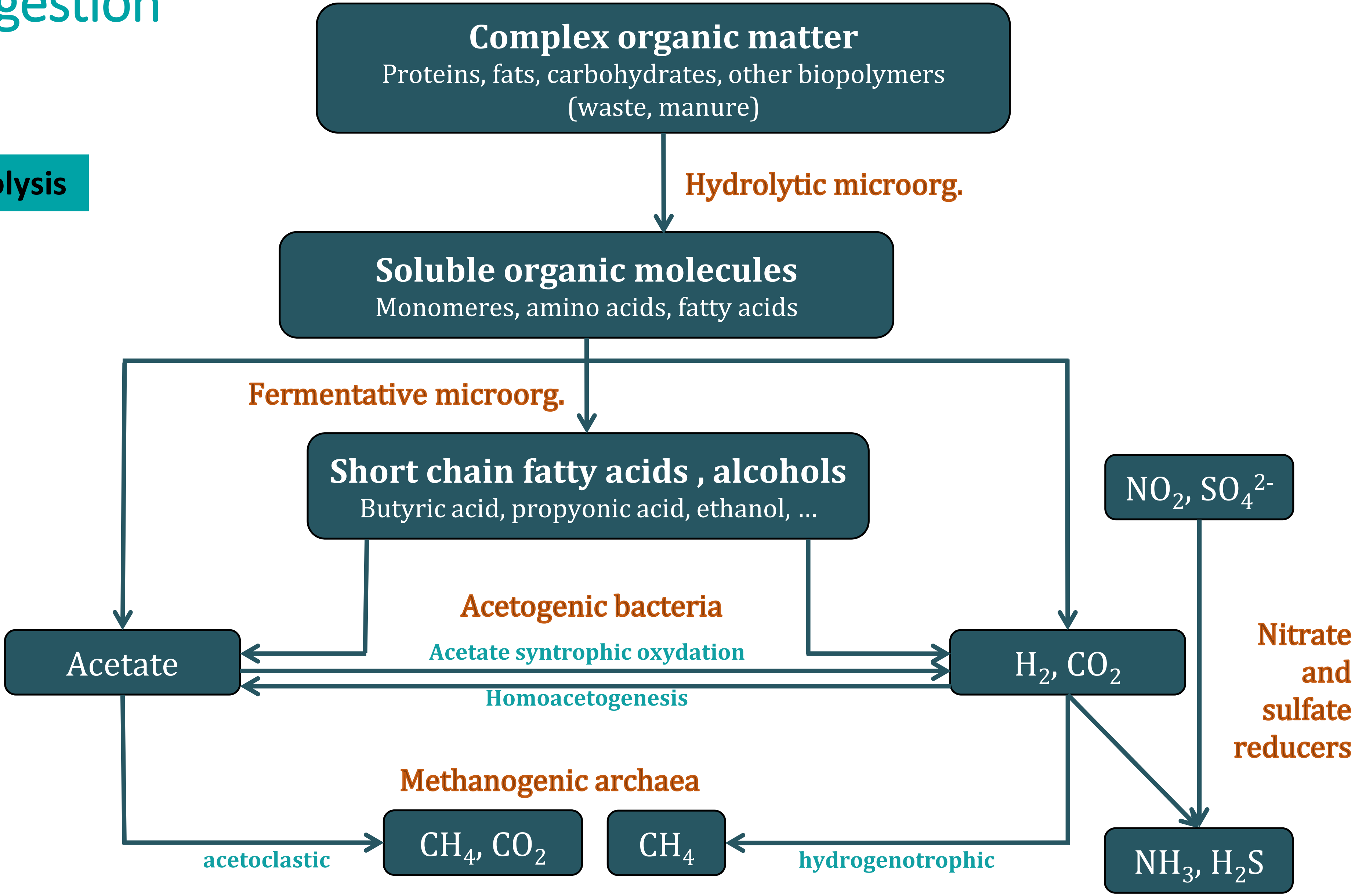
4 steps

Hydrolysis

Acidogenesis
= Fermentation

Acetogenesis

Methanogenesis



Complex organic matter
Proteins, fats, carbohydrates, other biopolymers
(waste, manure)

Hydrolytic microorg.

Soluble organic molecules
Monomeres, amino acids, fatty acids

Fermentative microorg.

Short chain fatty acids , alcohols
Butyric acid, propyonic acid, ethanol, …

NO_2, SO_4^{2-}

Acetogenic bacteria

Acetate

Acetate syntrophic oxydation

Homoacetogenesis

H_2, CO_2

Nitrate
and
sulfate
reducers

Methanogenic archaea

acetoclastic

CH_4, CO_2

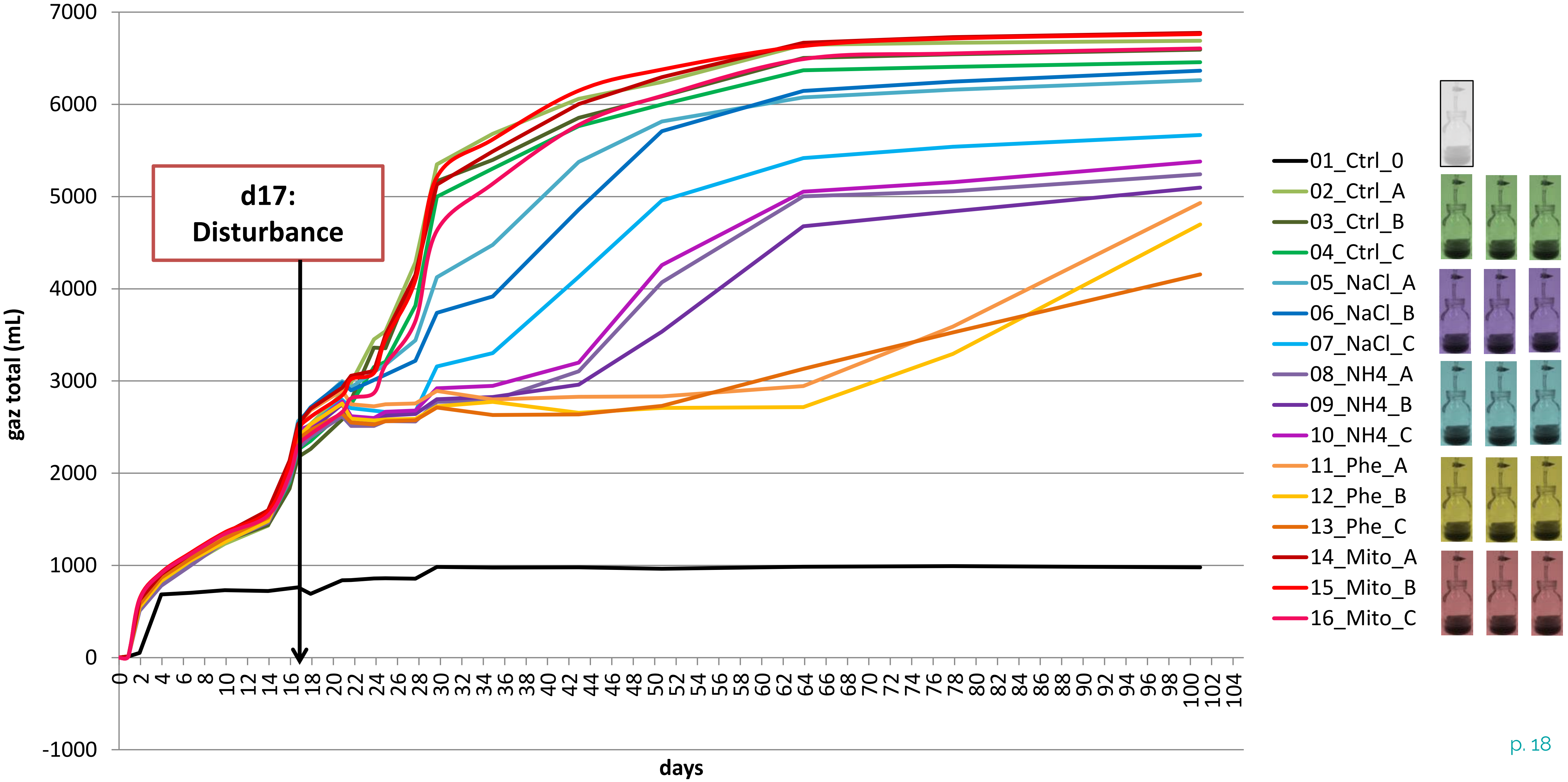
CH_4

hydrogenotrophic

NH_3, H_2S

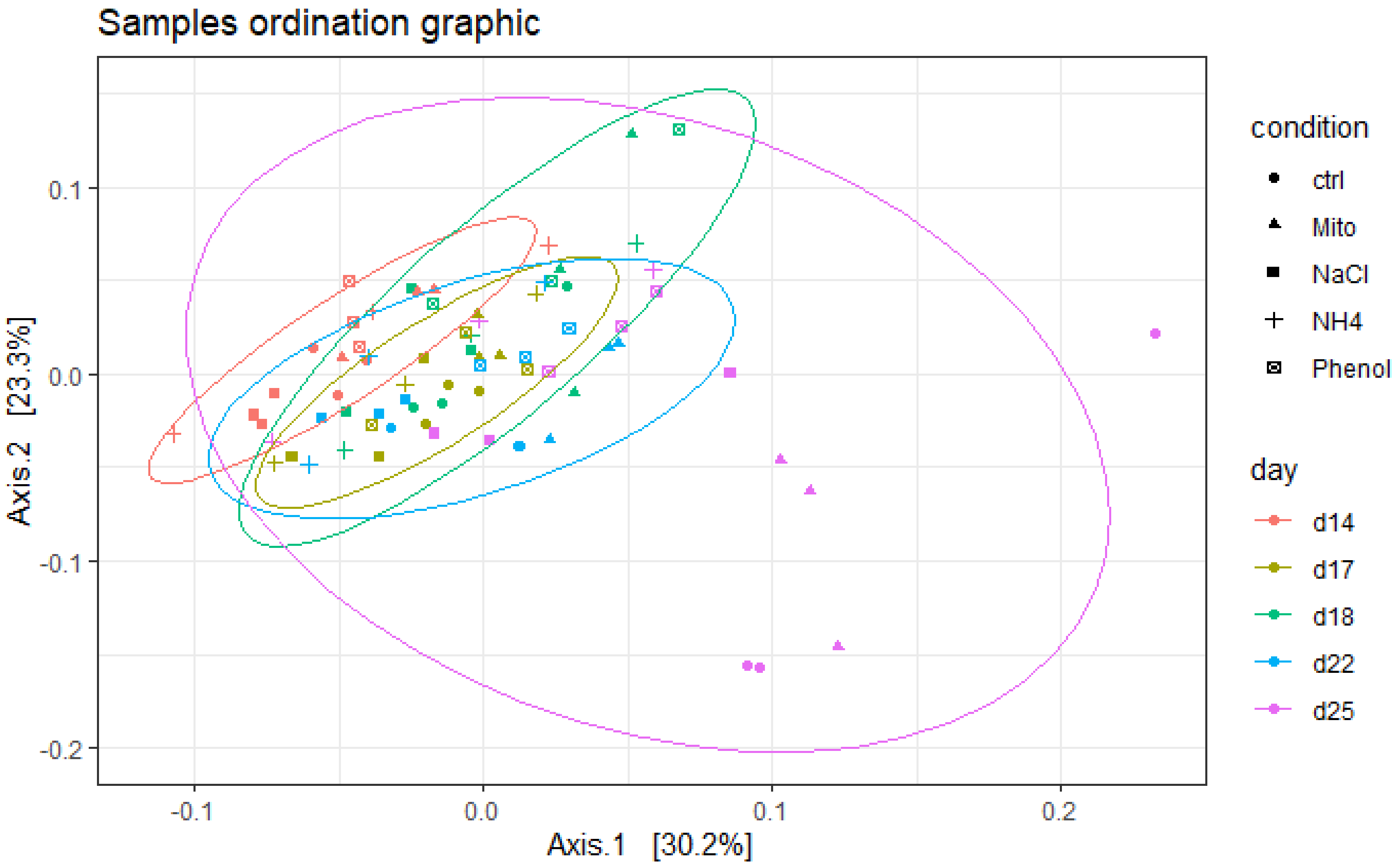
+ others pathways…

Impact on total cumulative gaz production ? YES



Impact on microbial community composition at the genus level?

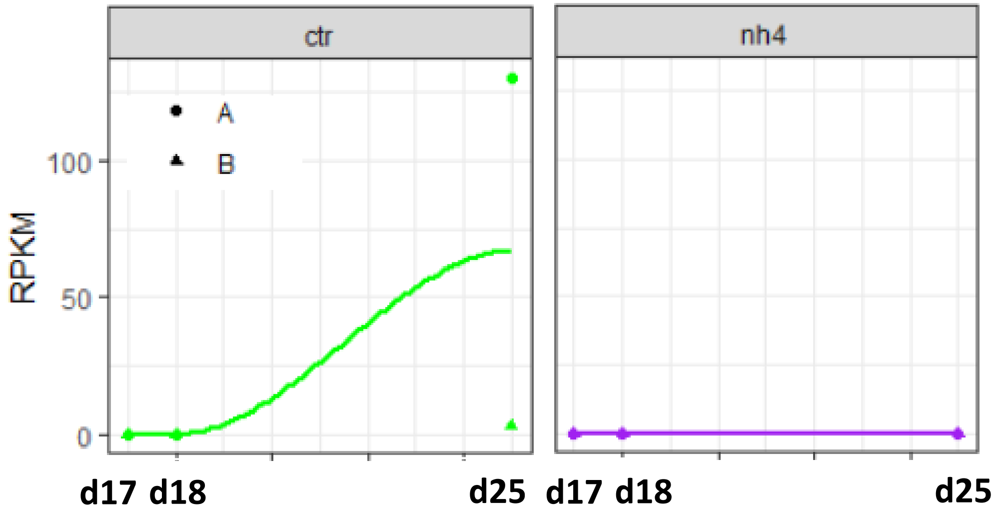
Beta diversity PCoA

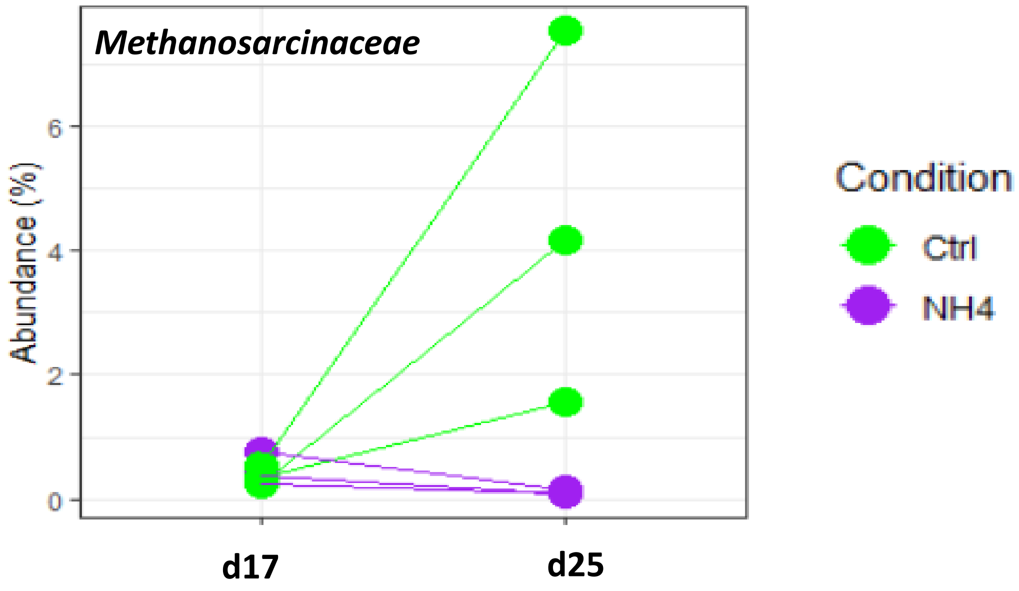


Variation particularly visible on day 25 when looking at the genus level

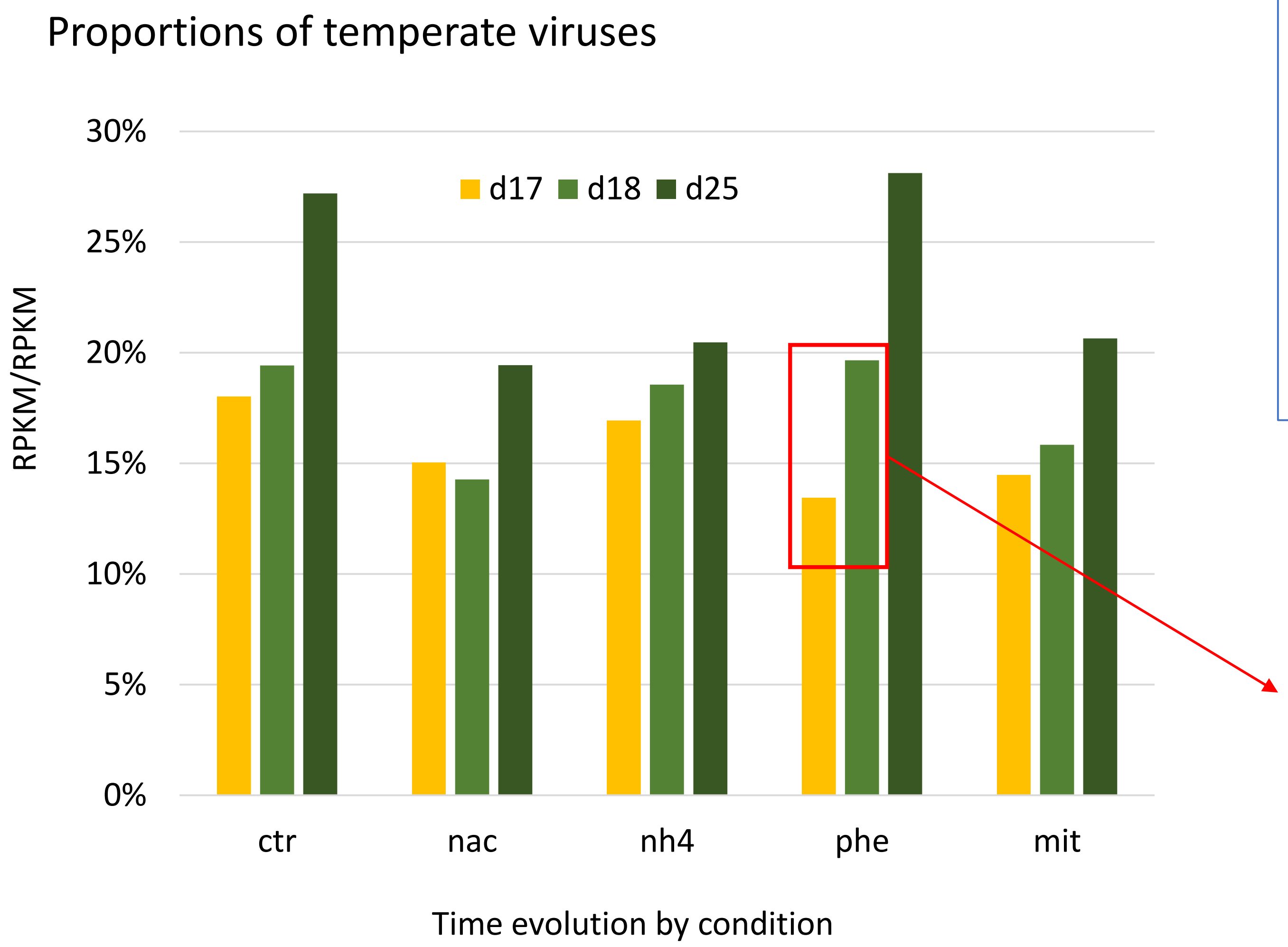
Abundance evolution of methanosarcinaceae

k127_68864 infecting Methanosarcinaceae





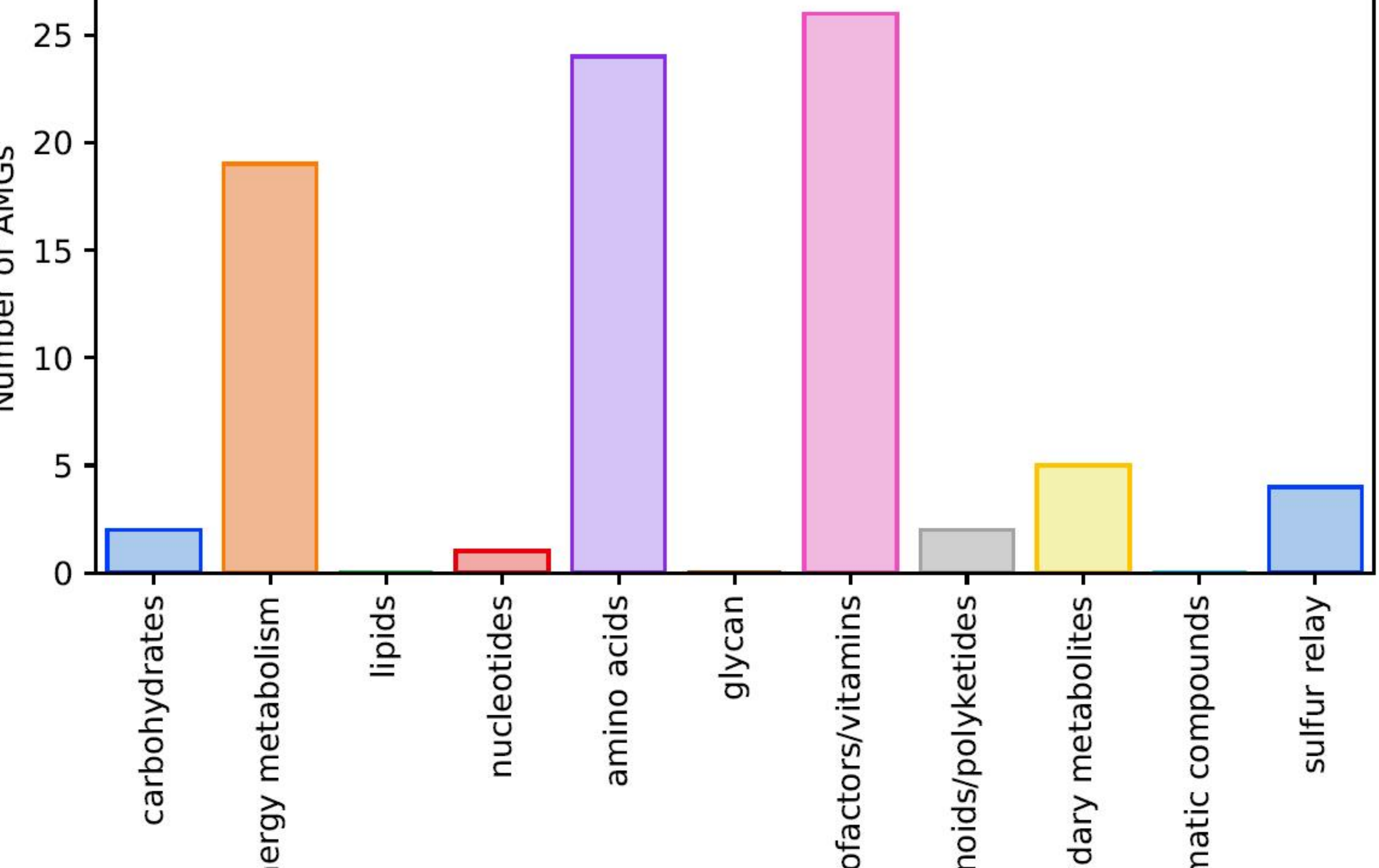
What about temperate viruses ?



<u>Time-bomb hypothesis</u>

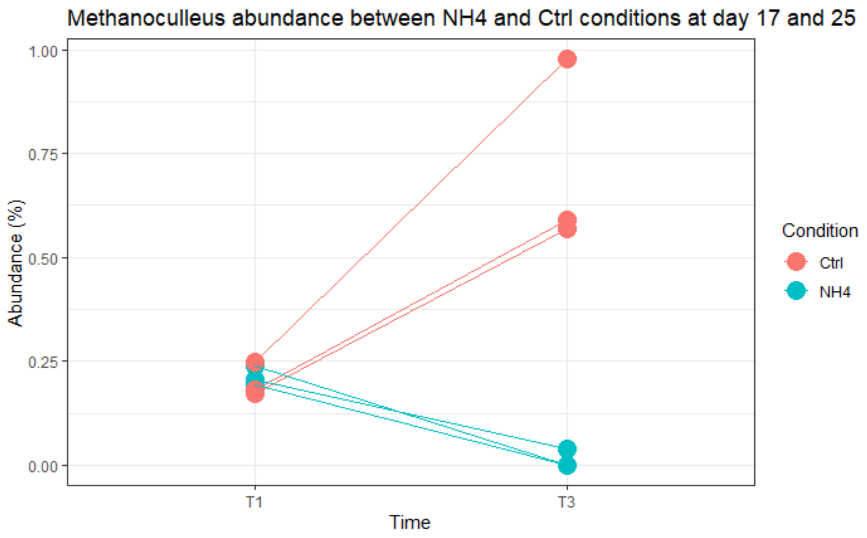
Stress could increase the proportion of particles from temperate viruses, by activating the lytic cycle of proviruses.

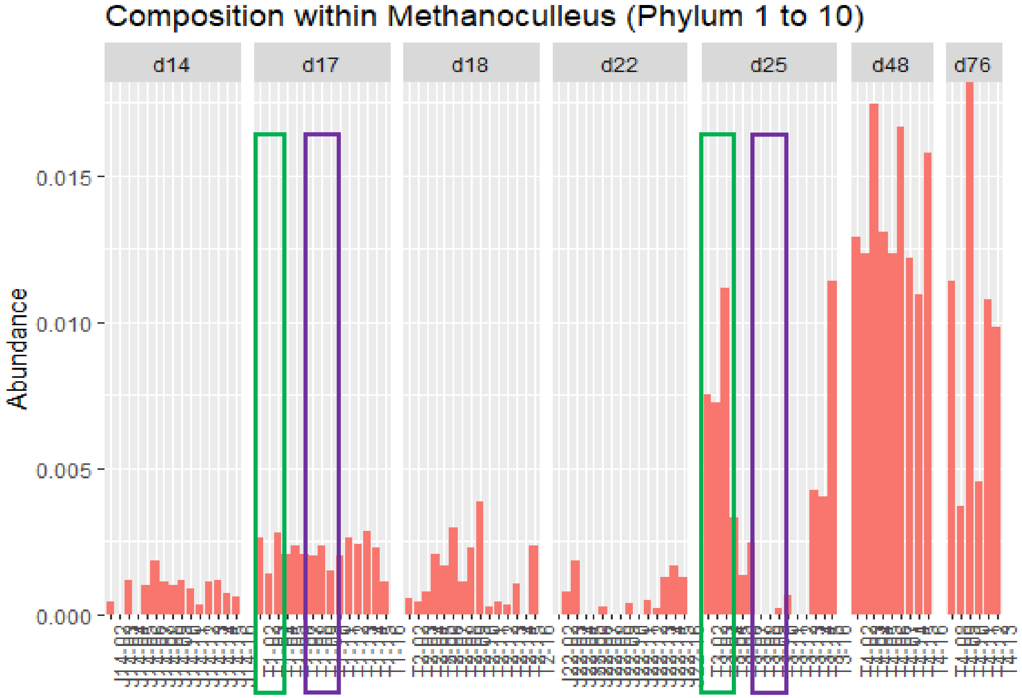
→ Looking further into the induction of prophages in phenol disturbance experiment could be interesting

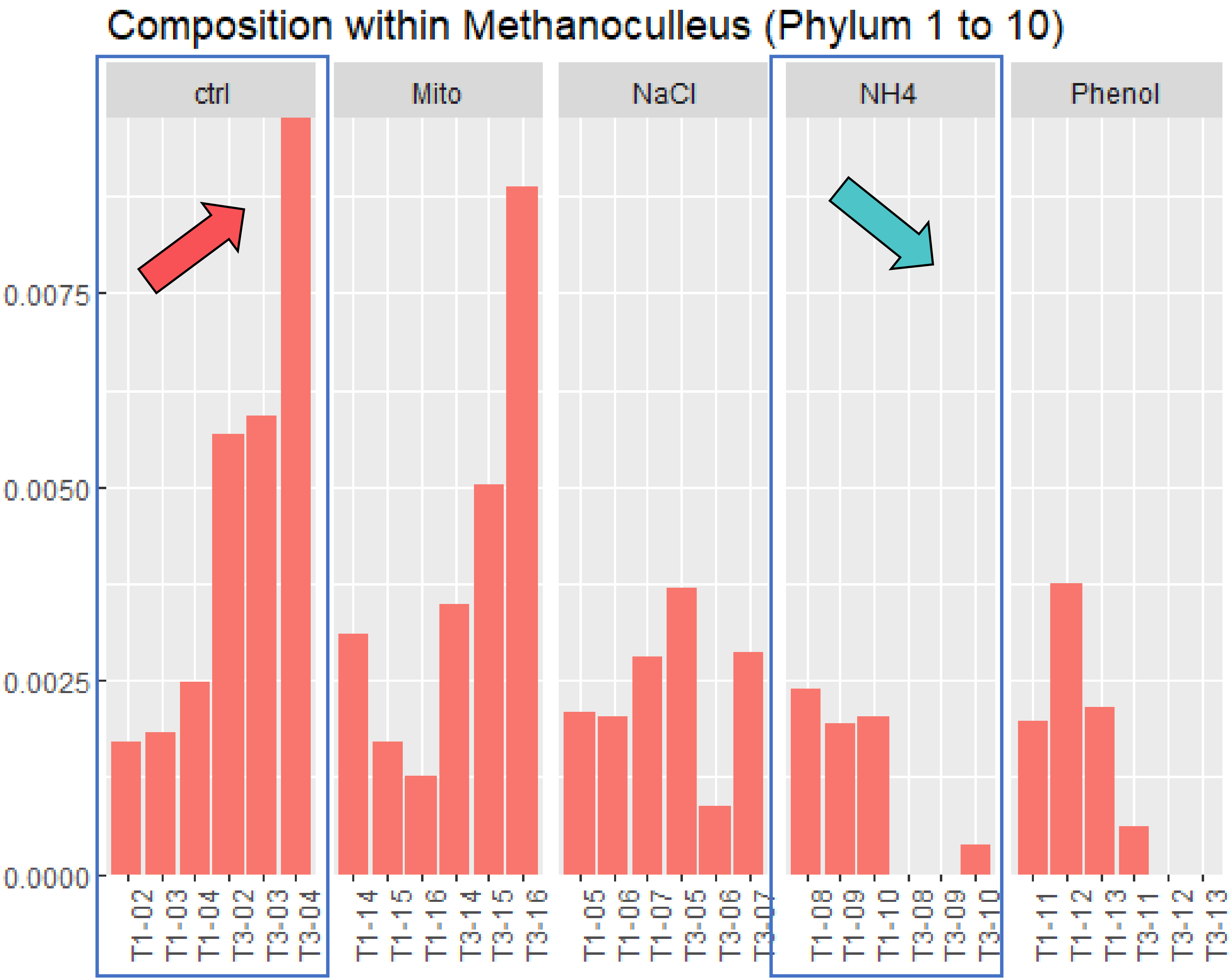

Number of AMGs
25
20
15
10
5
0
carbohydrates
ergy metabolism
lipids
nucleotides
amino acids
glycan
ofactors/vitamins
noids/polyketides
dary metabolites
matic compounds
sulfur relay

Methanoculleus

Log2FoldChange : -6.817845

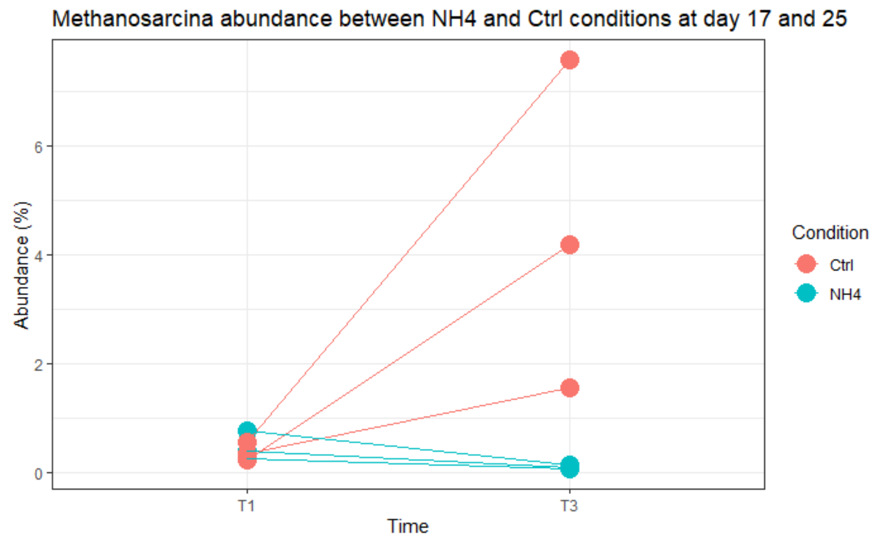

Methanoculleus abundance between NH4 and Ctrl conditions at day 17 and 25

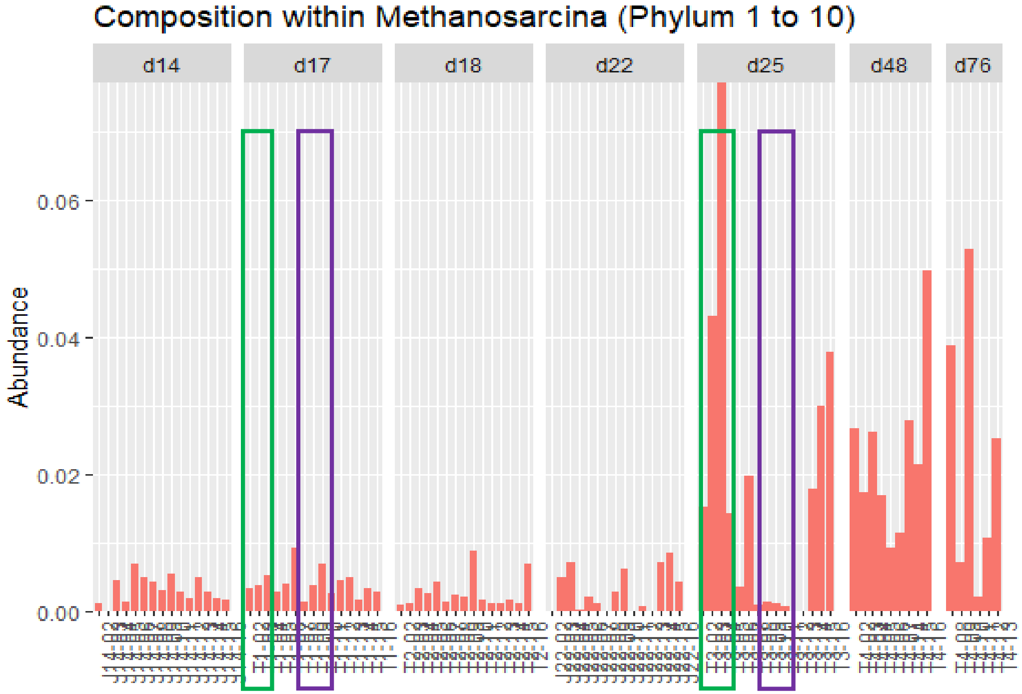

Composition within Methanoculleus (Phylum 1 to 10)

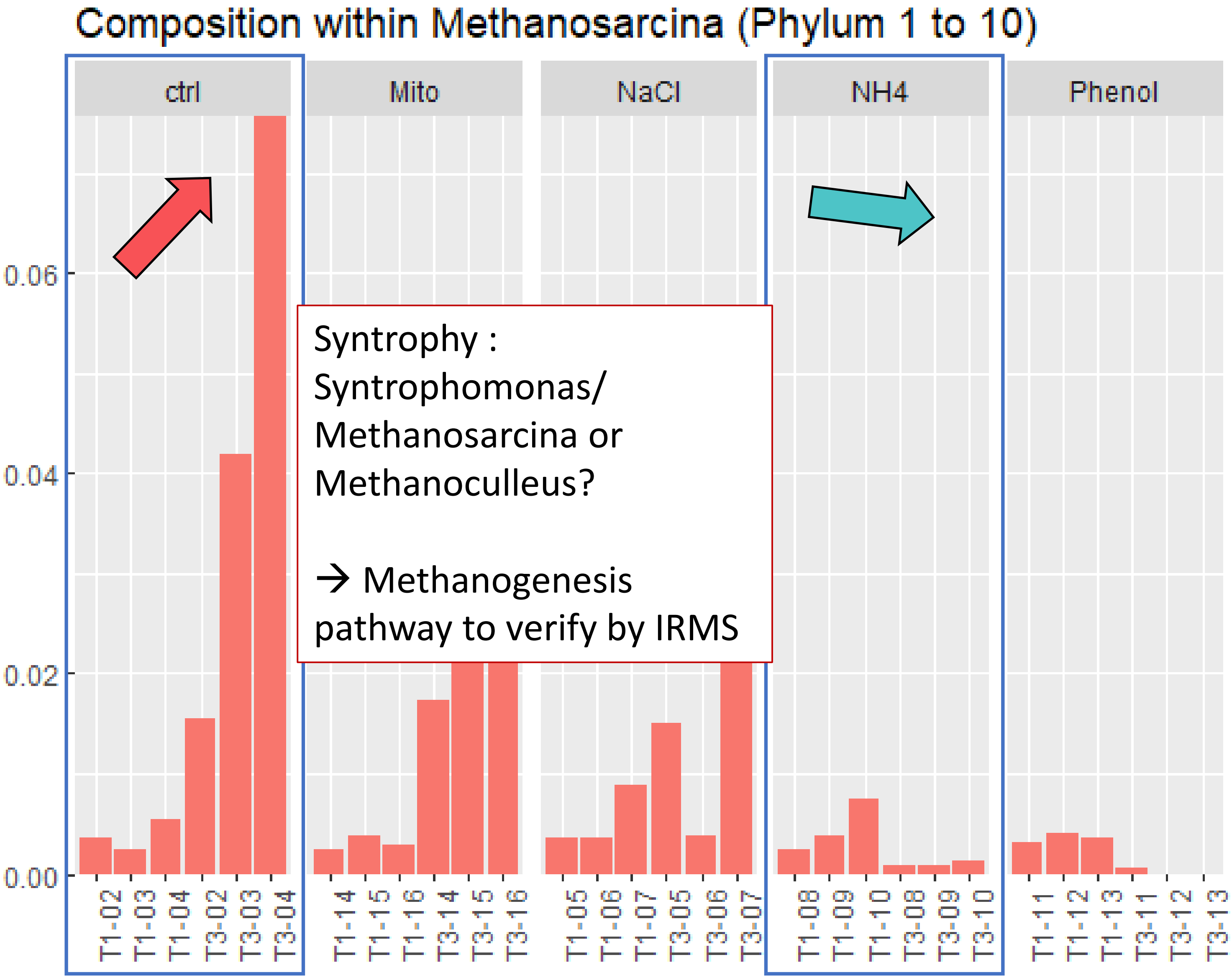

Composition within Methanoculleus (Phylum 1 to 10)

Methanosarcina

Log2FoldChange : -5.332194







Syntrophy :
Syntrophomonas/
Methanosarcina or
Methanoculleus?

→ Methanogenesis pathway to verify by IRMS