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



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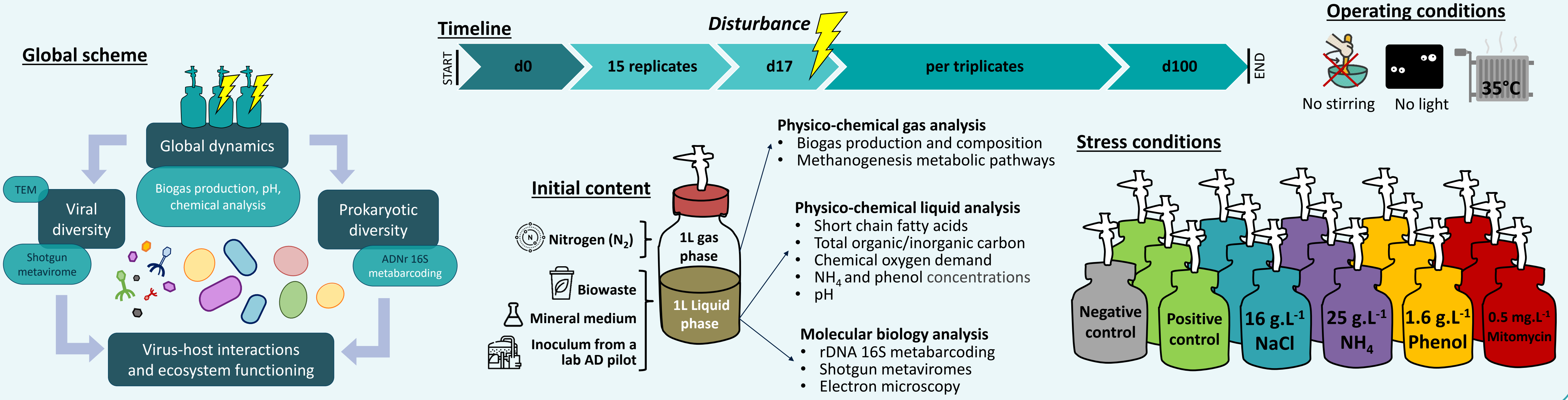
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## Context

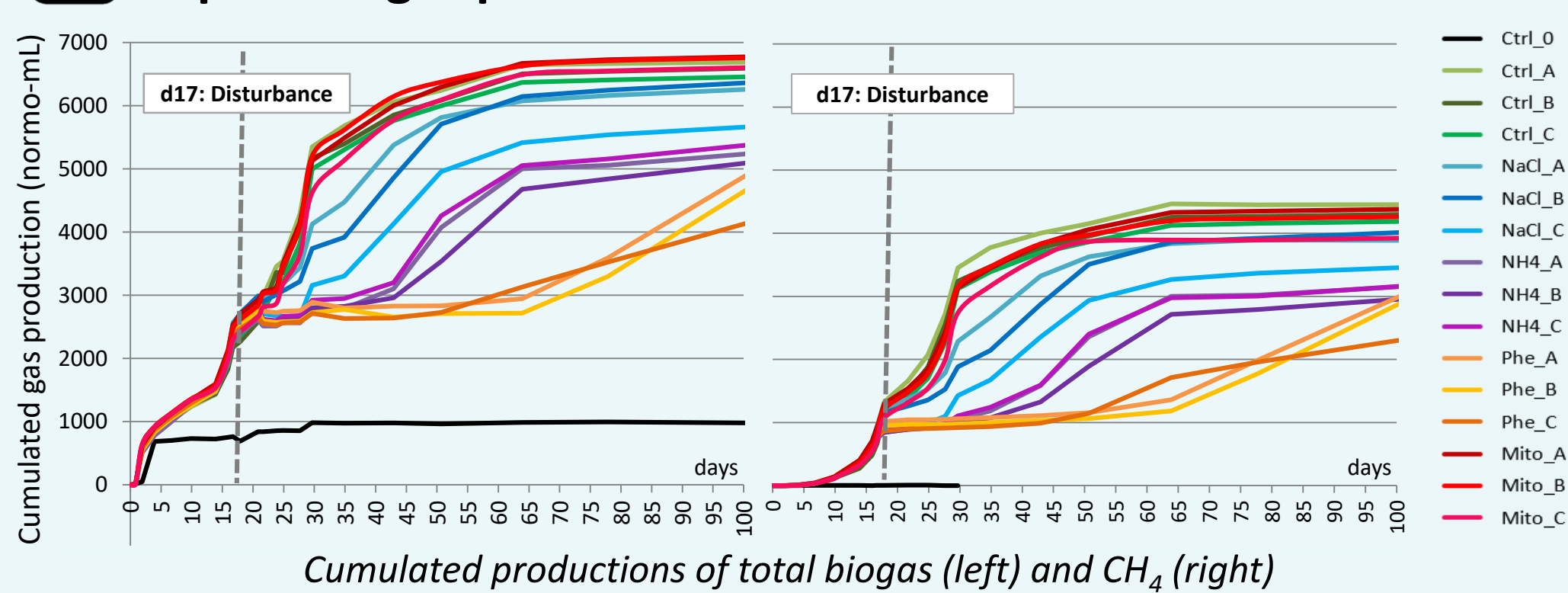
Viruses of microbes are major players in various ecosystems, typically affecting the structuration and dynamics of microbial communities [1]. They nevertheless remain poorly explored in anaerobic digestion (AD) plants [2], where biowaste is valorised into methane-rich biogas. The AD process ecosystems are very sensitive to disturbance, leading to inhibition and loss of methane production [3]. We were therefore interested in better understanding the interplay between abiotic disturbance, microbial community composition, including the viromes, and process performance.

## Experimental design

The variations of both viral and prokaryotic populations were monitored in batch AD microcosms under abiotic stress, during a hundred days. Four distinct abiotic disturbances were tested in triplicates: during incubation, either NaCl, NH<sub>4</sub>Cl or phenol was injected into the reactors, as inhibitors previously reported in full-scale plants. Mitomycin C was also tested, since it can induce the activation of lytic cycle for proviruses.



## Impact on gas production



→ We confirmed a significant impact of the tested stresses on biogas production, although with variable intensity

## DESeq model

Time evolution comparison: 2 time points, 2 conditions

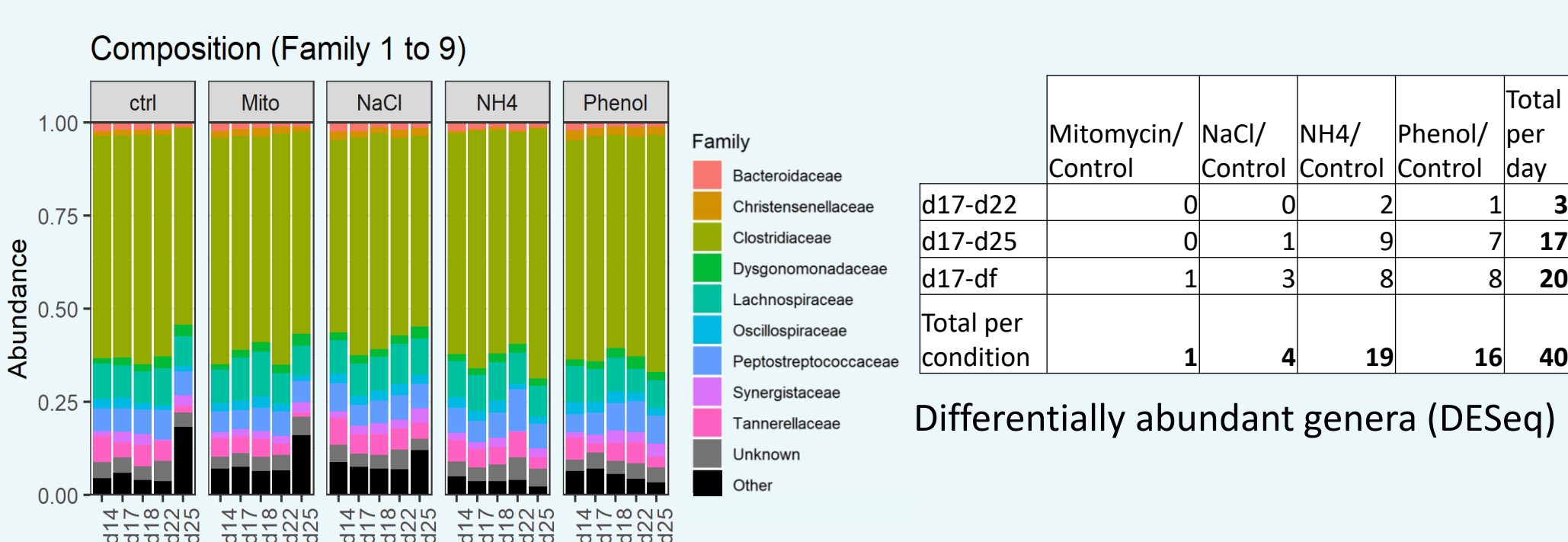
`model.matrix(~ condition + condition:replicate + condition:day, data)`

→ Identification of prokaryotic genera or viral contigs showing **differential evolutions** compared to the control.

Considering the high biological variability among replicates, the model took into account the intra-condition variability

## Microbial community dynamics

16S rDNA metabarcoding (Archaea + Bacteria, V4 region, Ion Torrent PGM)



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

→ No shift was visible at rank family but there was an impact at a finer level: 20 distinct genera presented significantly different dynamics

## Metaviromes

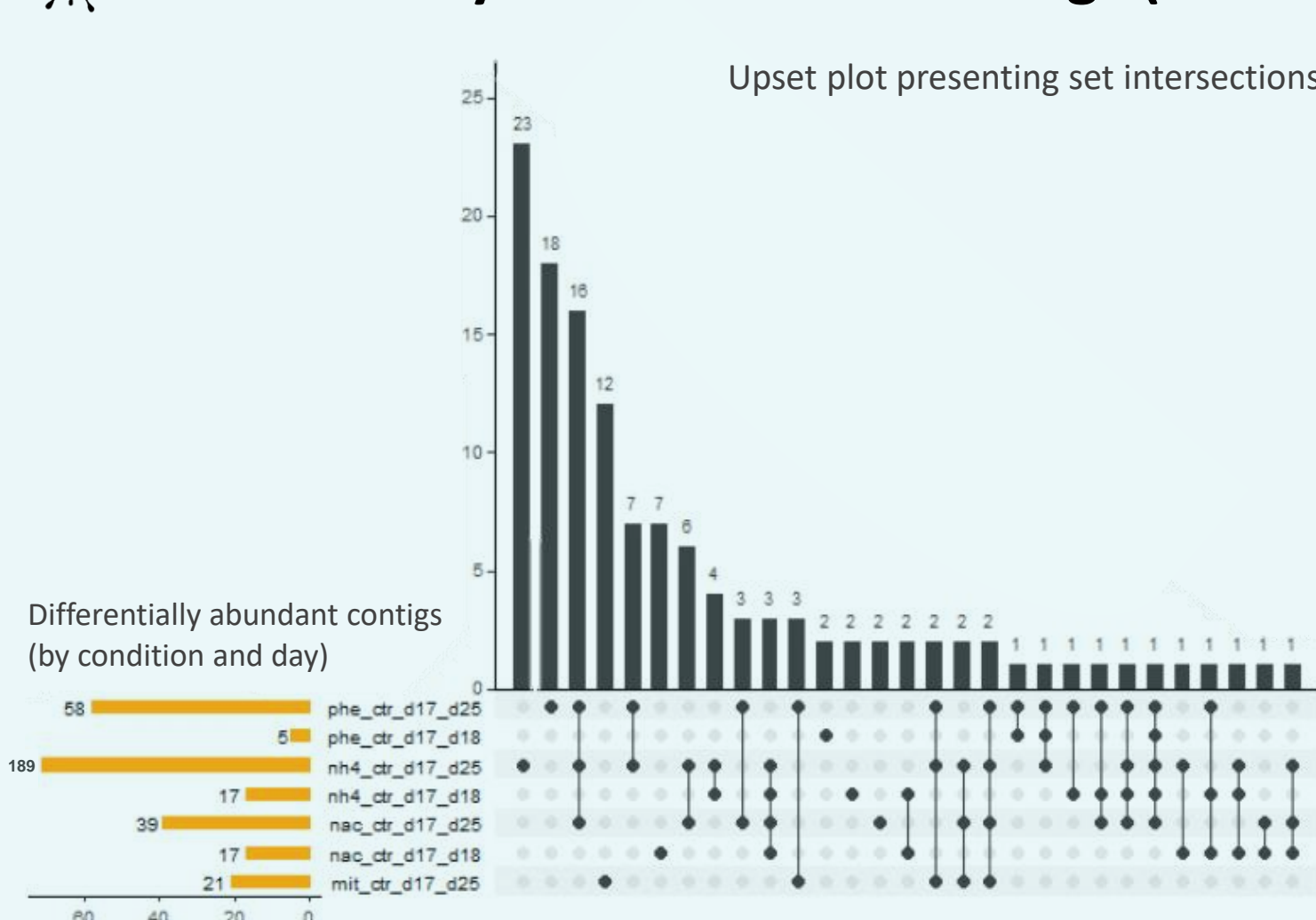
### Methods

- Shotgun sequencing of 30 selected metaviromes (Illumina, 2 x 150 bp)
- Coassembly (Megahit) with a snakemake pipeline developed in our lab [4]
- Virus detection with tools such as VIBRANT [5], CheckV [6] and VirSorter2 [6]

### Coassembly statistics

- 10<sup>5</sup> contigs longer than 1,000 bases
- N50 = 3,886 bases
- 45,914 putative viral contigs (VIBRANT) including 2,815 with the status "complete"
- 430 highly reliable viral contigs (detected by at least two distinct tools)
- 64,10<sup>6</sup> mapped reads per sample on average (representing 97.5% of the reads)

## Differentially abundant viral contigs (DESeq)

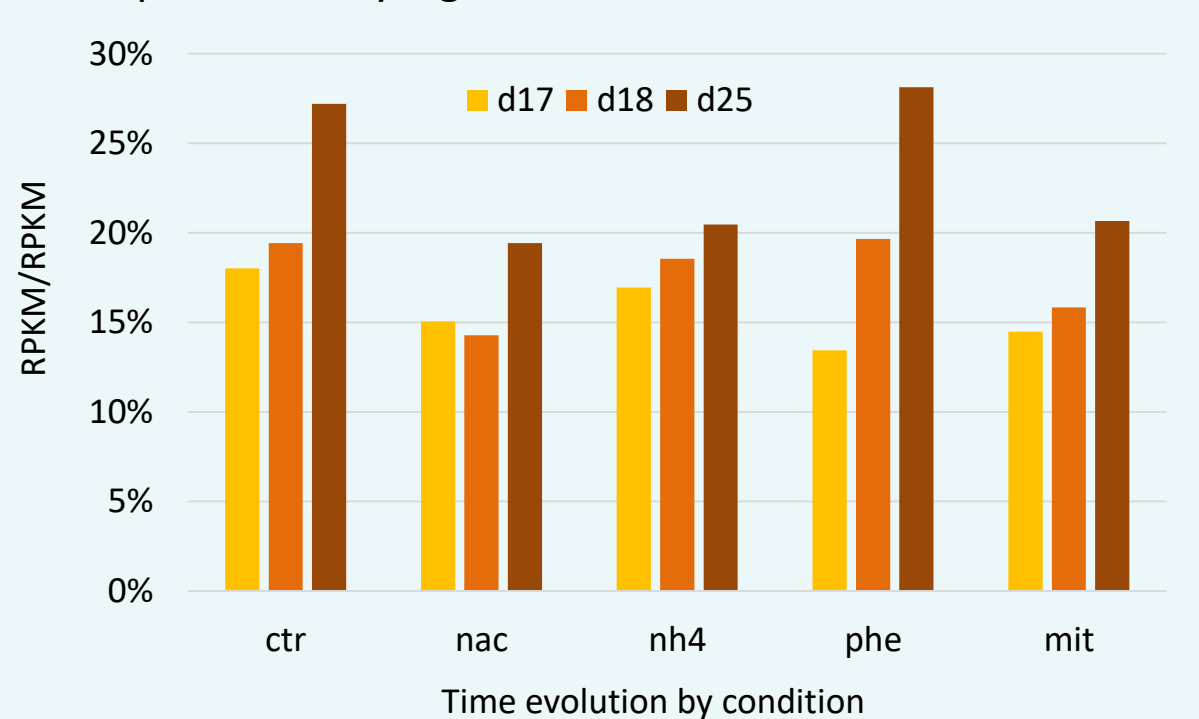


→ 127 viral contigs were differentially abundant when comparing time evolutions (before and after disturbance). 16 of them were common among conditions NaCl, NH<sub>4</sub> and Phenol on day 25

### Time-bomb hypothesis [8]

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

### Proportions of lysogenic viruses



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

## Conclusions

- ✓ The significant impact of the tested stresses on biogas production was confirmed
- ✓ Microbial community's shifts were more significant on day 25
- ✓ 127 viral contigs were differentially abundant

## Perspectives

- Analysis of viral contigs of interest (host prediction, taxonomic affiliation, gene content)
- Auxiliary metabolic genes (AMG) will be particularly interesting to study further

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