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► **To cite this version:**

Marion Coves, Laurent Mazéas, Olivier Chapleur, Cédric Midoux, Ludwig E Jardillier, et al.. Characterization of virus-host dynamics in anaerobic digesters under abiotic stress. *Viruses of Microbes*, Jul 2022, Braga, Portugal. . hal-04356367

HAL Id: hal-04356367

<https://hal.inrae.fr/hal-04356367v1>

Submitted on 20 Dec 2023

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



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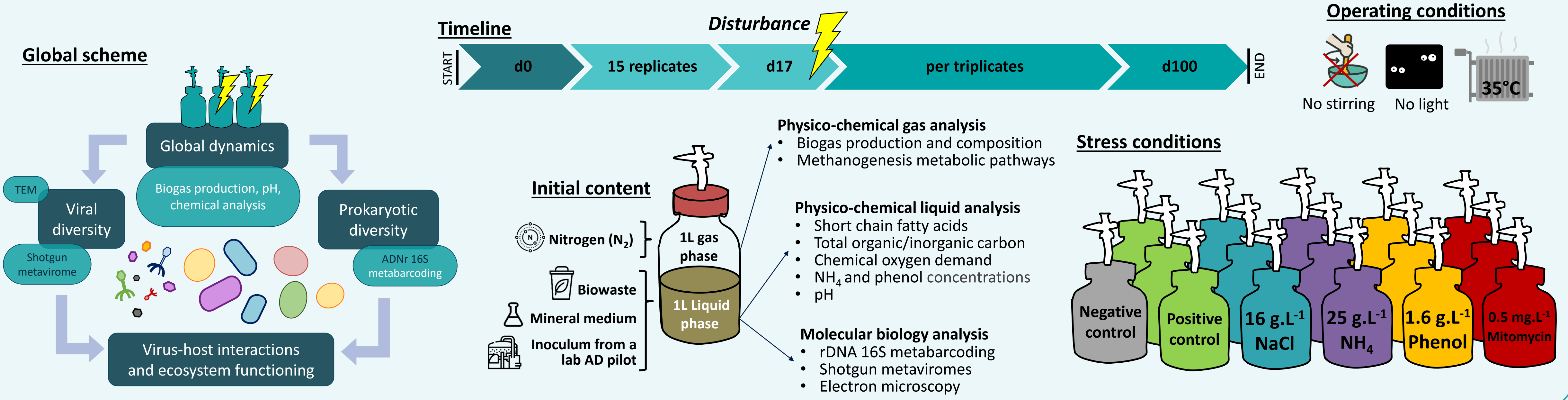
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Context

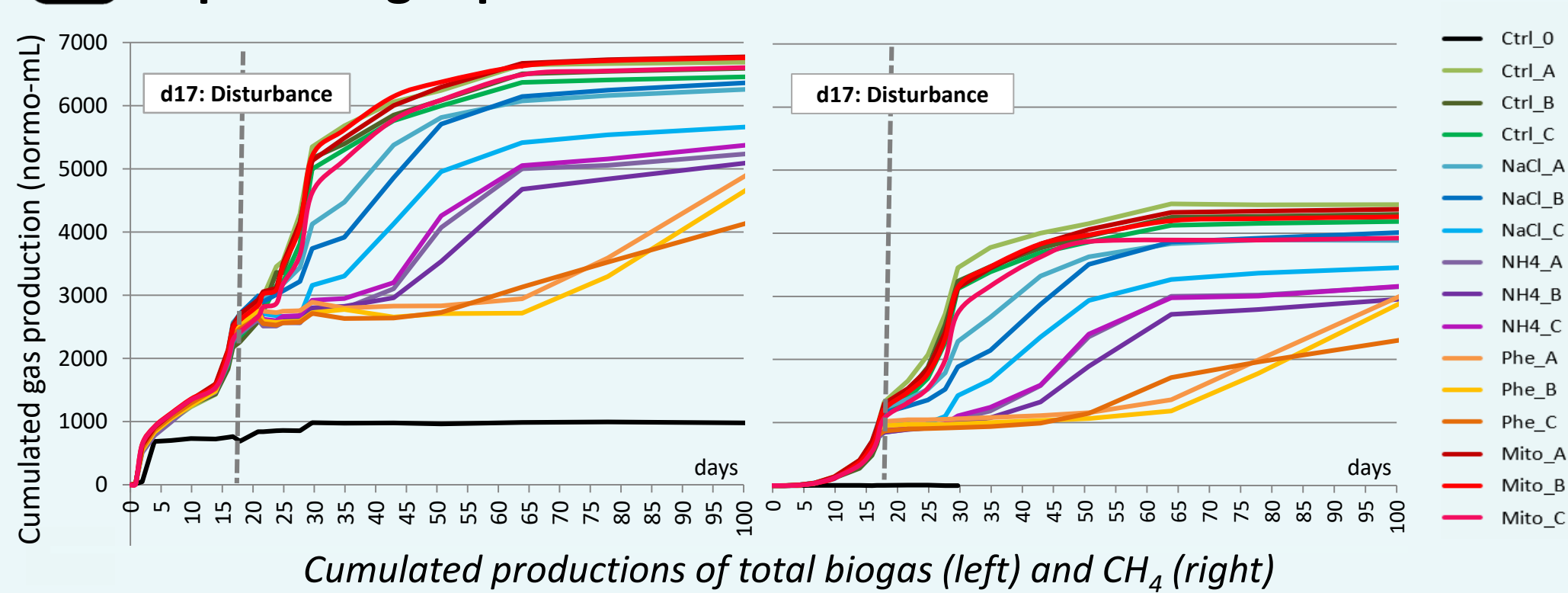
Viruses of microbes are major players in various ecosystems, typically affecting the structuration and dynamics of microbial communities [1]. They nevertheless remain poorly explored in anaerobic digestion (AD) plants [2], where biowaste is valorised into methane-rich biogas. The AD process ecosystems 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, NH₄Cl 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

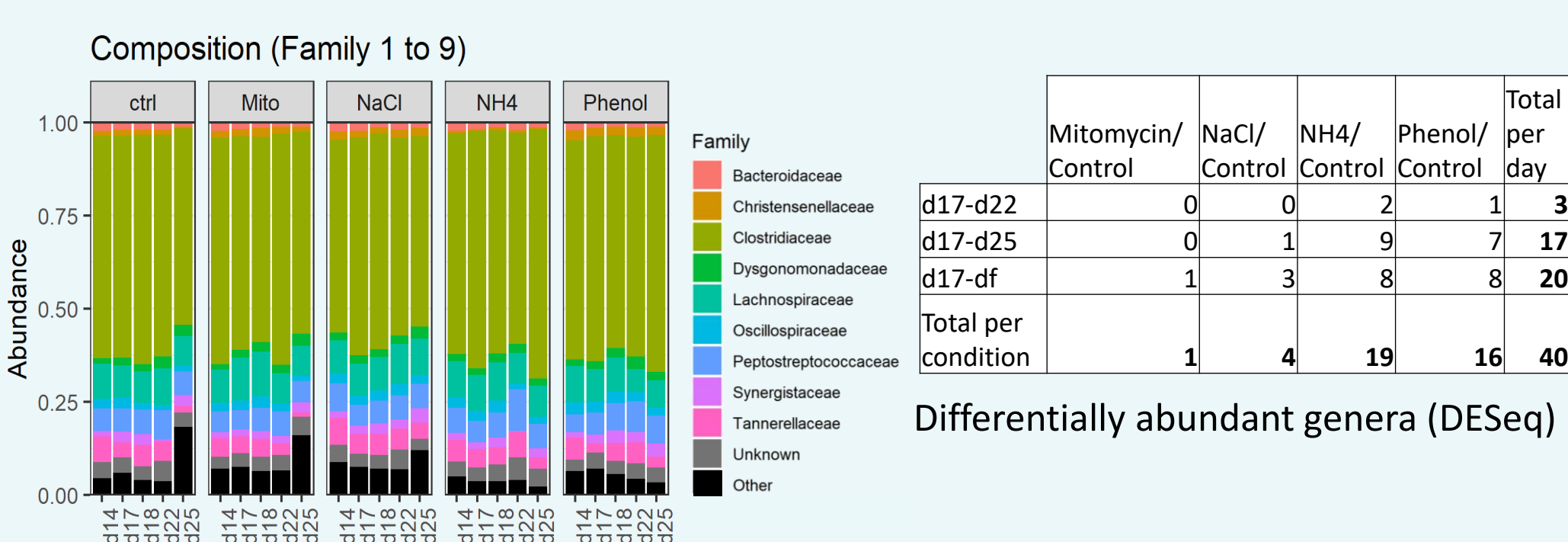
`model.matrix(~ condition + condition:replicate + condition:day, data)`

→ 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

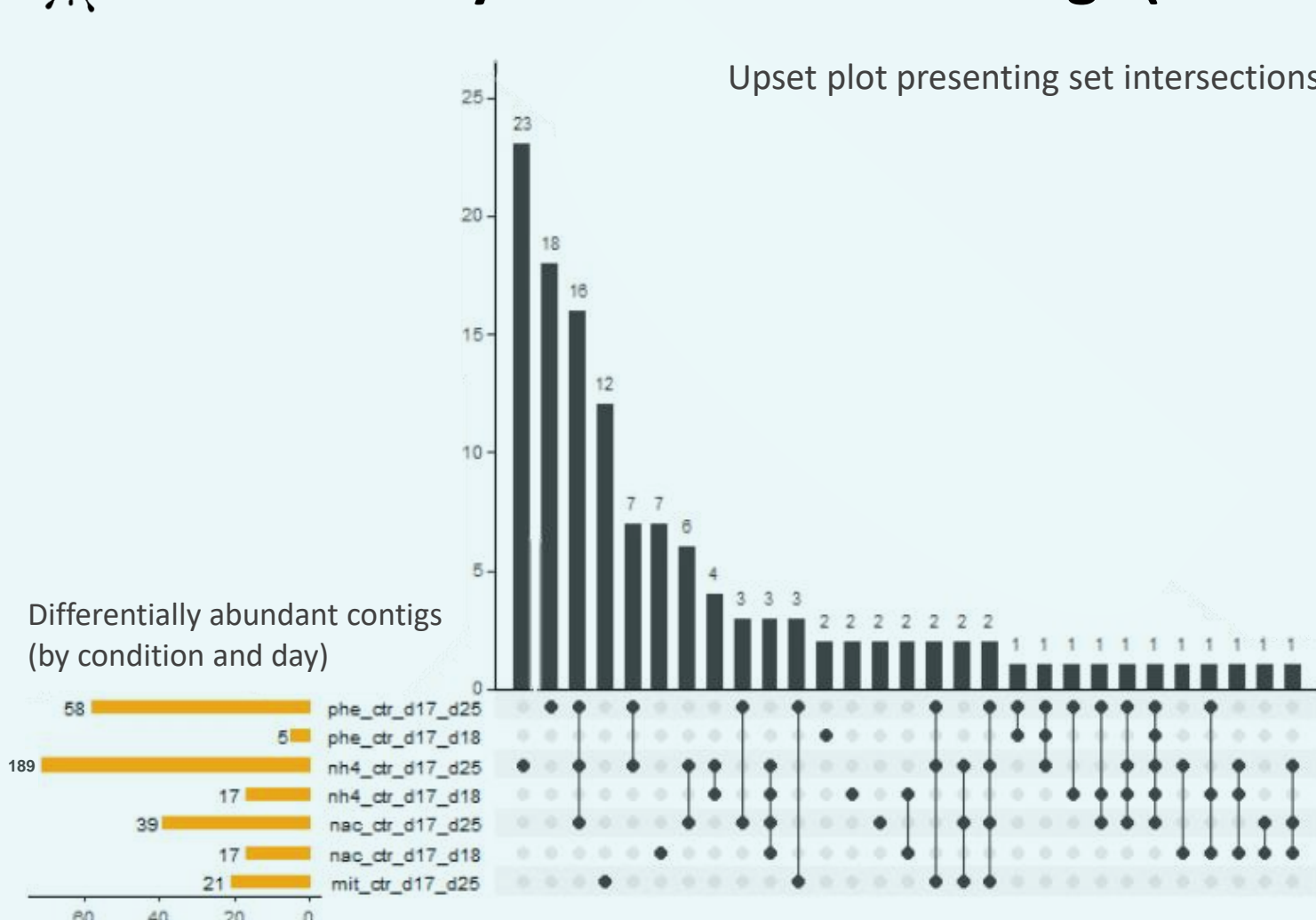
Methods

- 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

- 10⁵ 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)
- 64,10⁶ mapped reads per sample on average (representing 97.5% of the reads)

Differentially abundant viral contigs (DESeq)

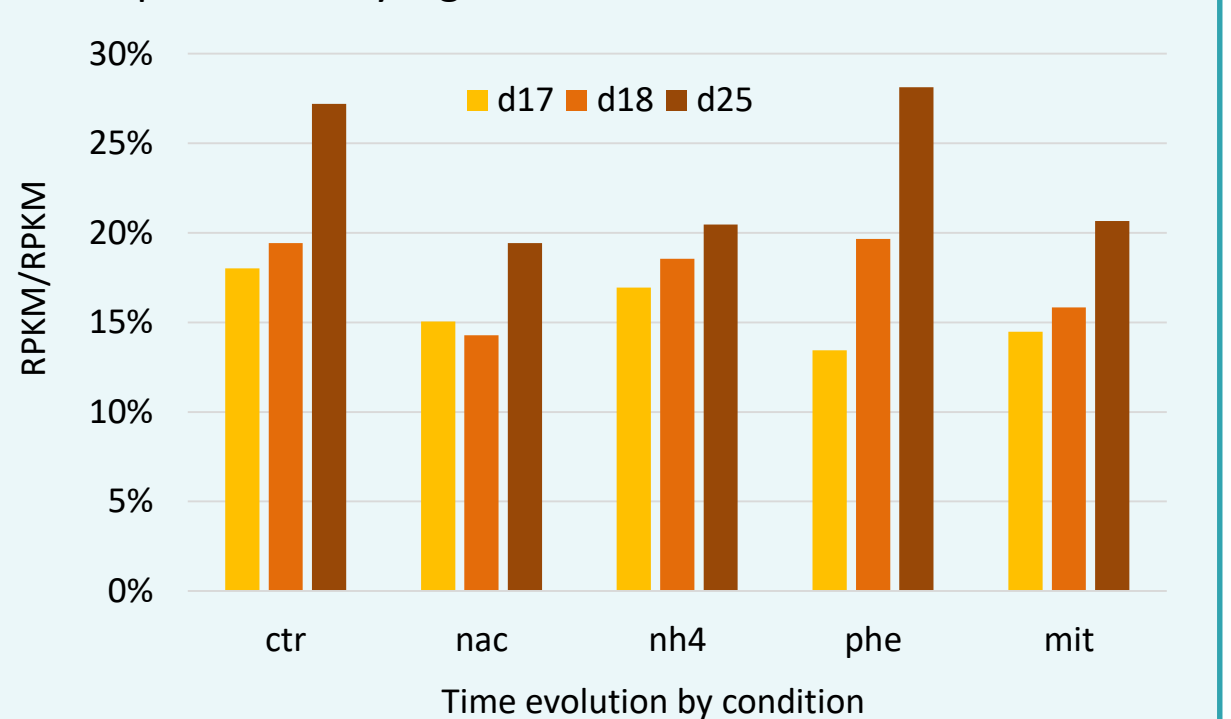


→ 127 viral contigs were differentially abundant when comparing time evolutions (before and after disturbance). 16 of them were common among conditions NaCl, NH₄ and Phenol on day 25

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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