



Time off feed affects enteric methane and rumen microbiome in sheep

ATF & EAAP Livestock Farming systems (LFS) study commission
EAAP Annual Meeting - Porto, Portugal - Monday 5th September 2022

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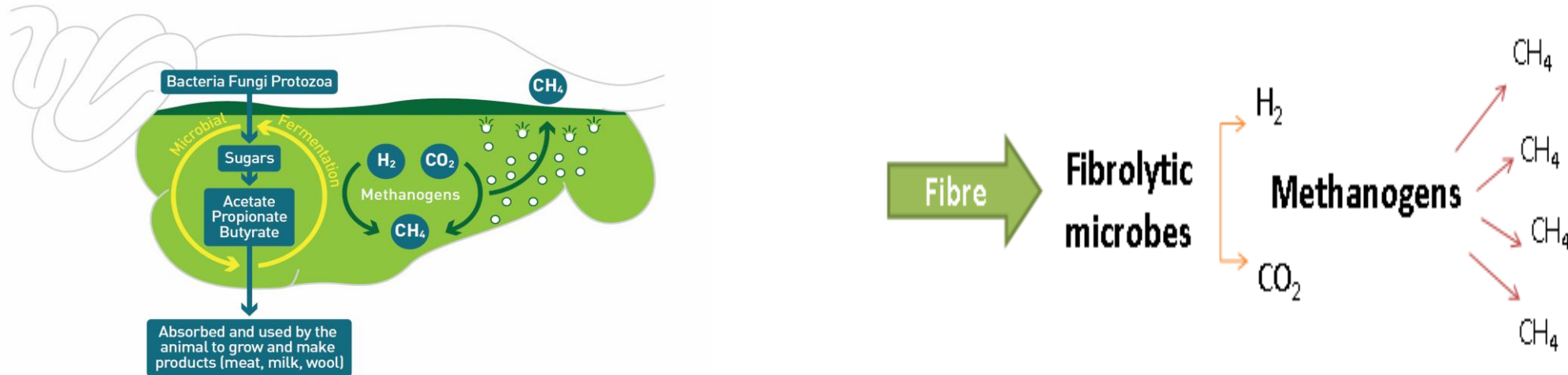
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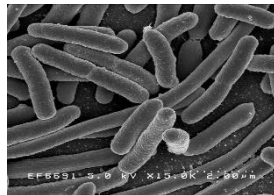
The gut microbiome in ruminants

Ruminants - unique in their ability to convert cellulose in plant cell walls into high quality meat and milk protein for humans



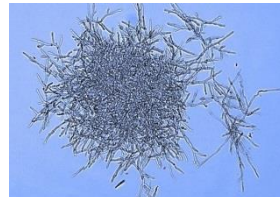
Bacteria

10¹⁰ to 10¹¹ cells/ml



Anaerobic Fungi

<10⁵ cells/ml



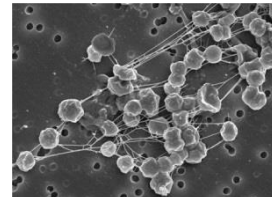
Ciliate Protozoa

<10⁵ cells/ml



Methanogenic Archaea

10⁶ to 10⁸ cells/ml



Viruses

10 phage for every bacteria



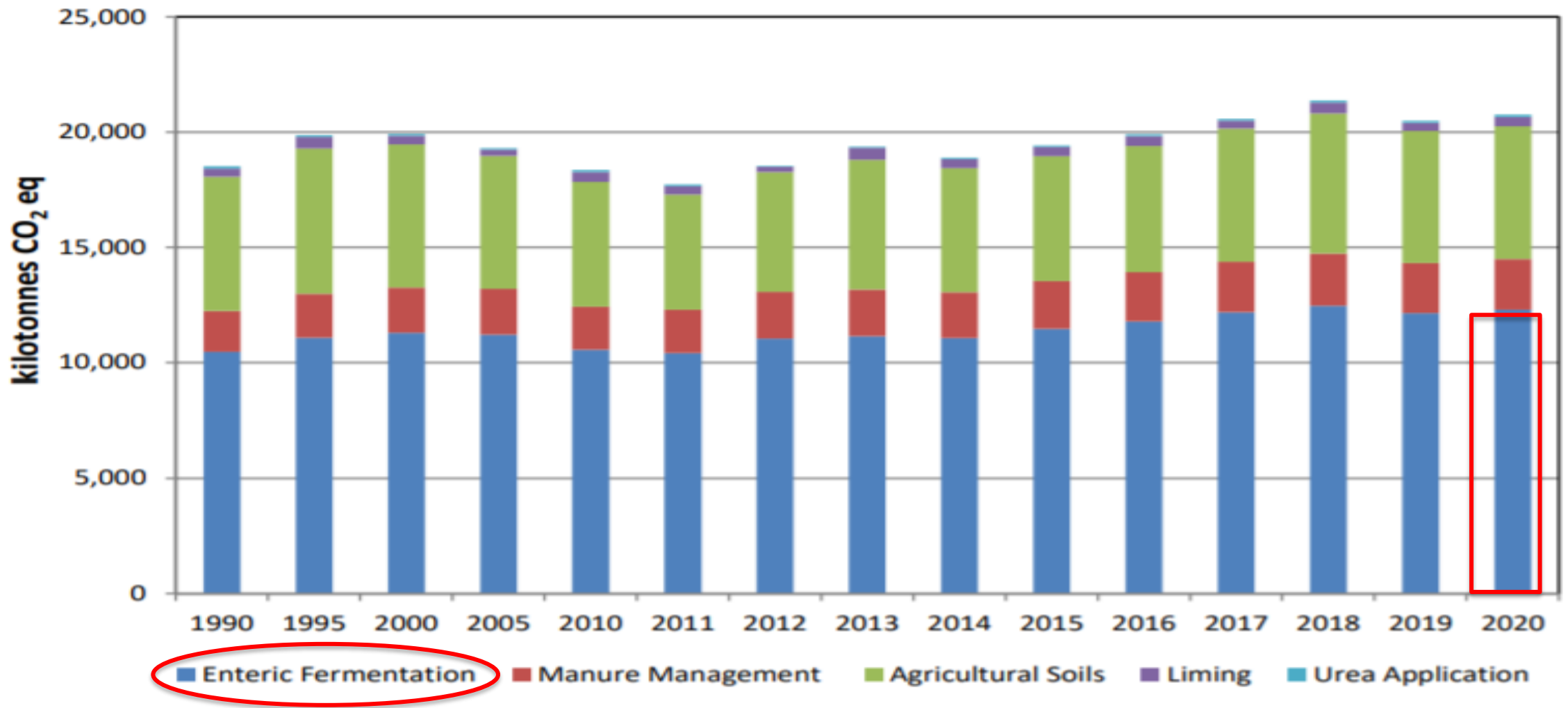
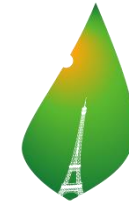


Figure 5.1 Total Emissions from Agriculture by Sector, 1990-2020

90-95%

International and national mitigation commitments

- COP 21 (UNFCCC Paris Agreement)
 - International commitment aiming to limit global warming to well below 2°C and pursuing efforts to limit it to 1.5°C
- EU 2030 – reduce GHG by 40% based on 1990 levels
Reduce GHG emissions by 55%, 2030 (EU fit for 55)
- “Ag Climatise” Government document (2019)
Climate neutral by 2050
- **Climate action plan 2021**
 - **25% reduction in agricultural GHG emissions**



PARIS2015
UN CLIMATE CHANGE CONFERENCE
COP21·CMP11



An Roinn Talmhaíochta,
Bia agus Mara
Department of Agriculture,
Food and the Marine



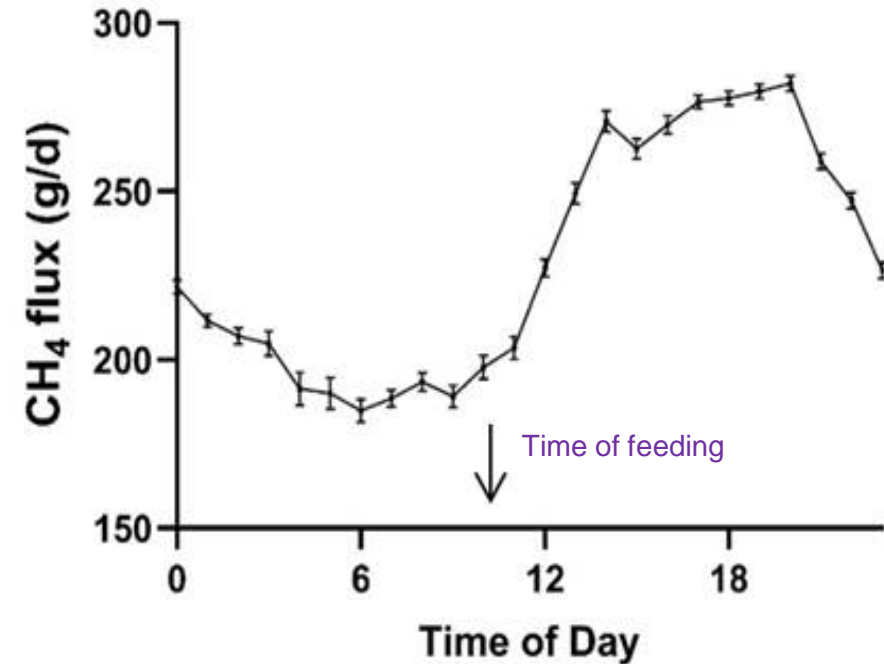
Importance of sheep to Irish economy

- **Sheep** production is an important agricultural enterprise in Ireland
 - Promoting biodiversity, economic sustainability to rural areas
- Contribution to Irish economy
 - 2019 - value €300 million
- Enteric CH₄ emissions sheep ~6% of national enteric CH₄
- More economical model for studying ruminal methanogenesis compared to cattle



Influence of diet on microbiome and CH₄ emissions

- Influence of diet on rumen microbiome (Carbury et al., 2012)
- Colonization of feed by rumen bacteria *in vitro* (Huws et al., 2016)
- Little data on effect of time off feed (TOF) on rumen microbiome and enteric CH₄ output of sheep



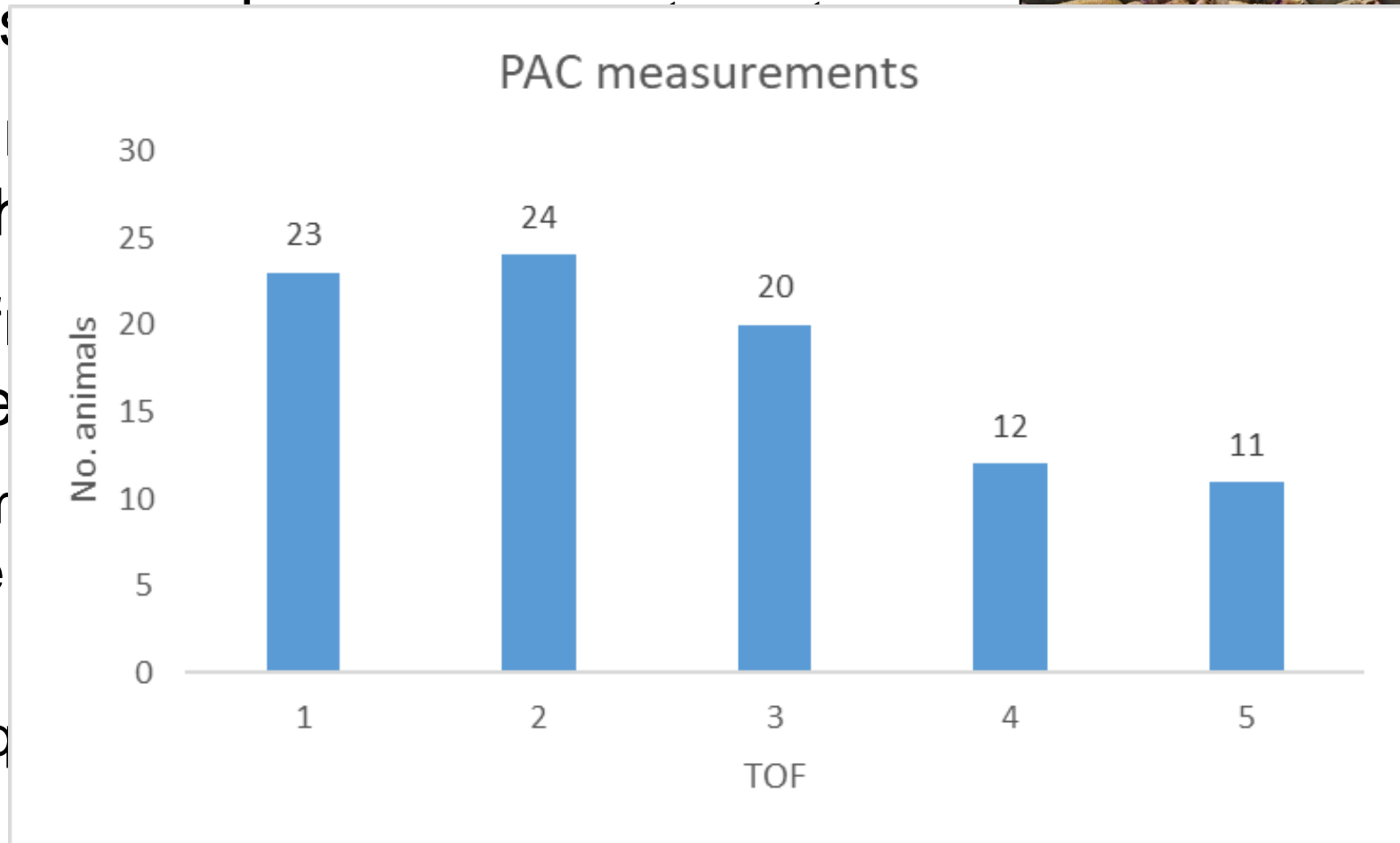
(Smith et al., 2021)

Objective

“Investigate the effect of time off feed on methane emissions and rumen microbiome in sheep”

Materials and methods

- 96 Belclare ewes
- Methane measurement using Accumulation Chamber
- Ewes removed from chamber for CH_4 measurement
 - Rumen Fluid sampled
 - PAC measurement apparatus
 - Snap frozen in liquid nitrogen

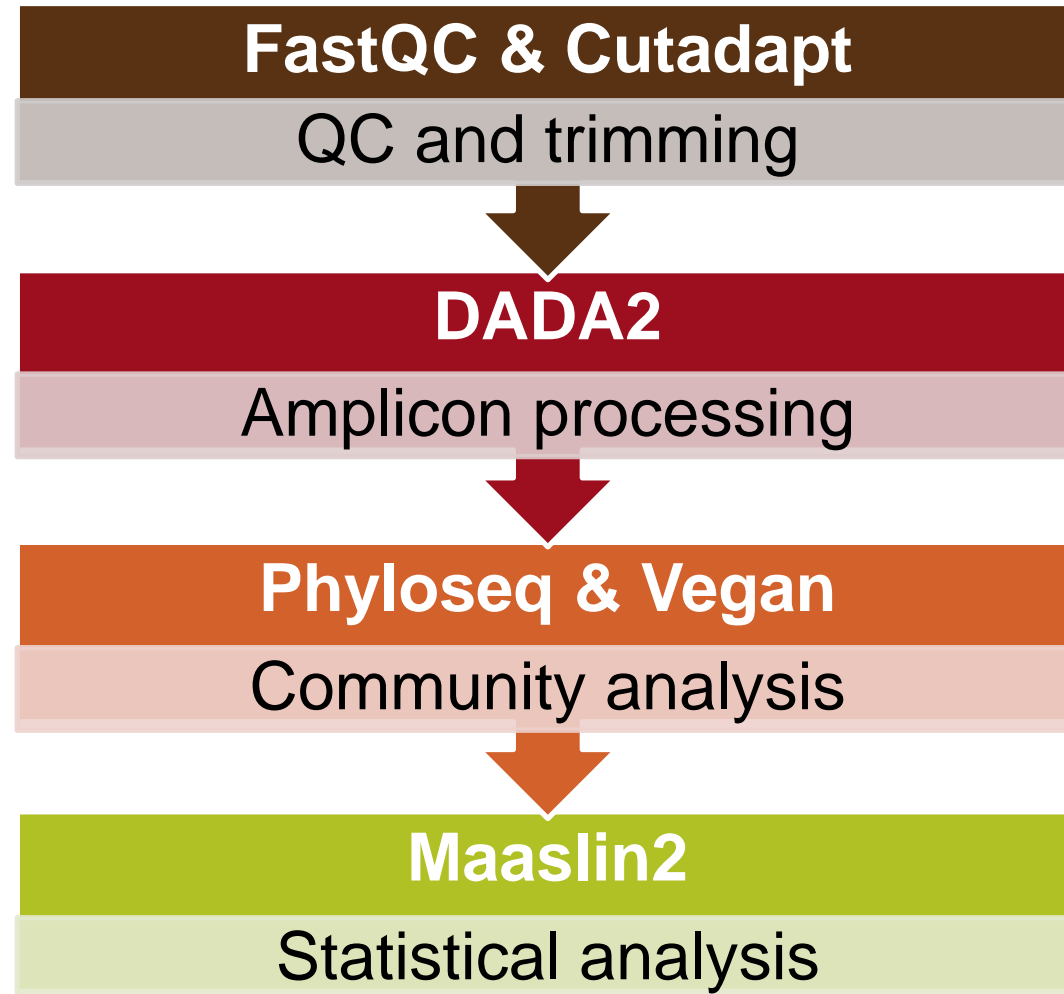


DNA/RNA extraction and sequencing

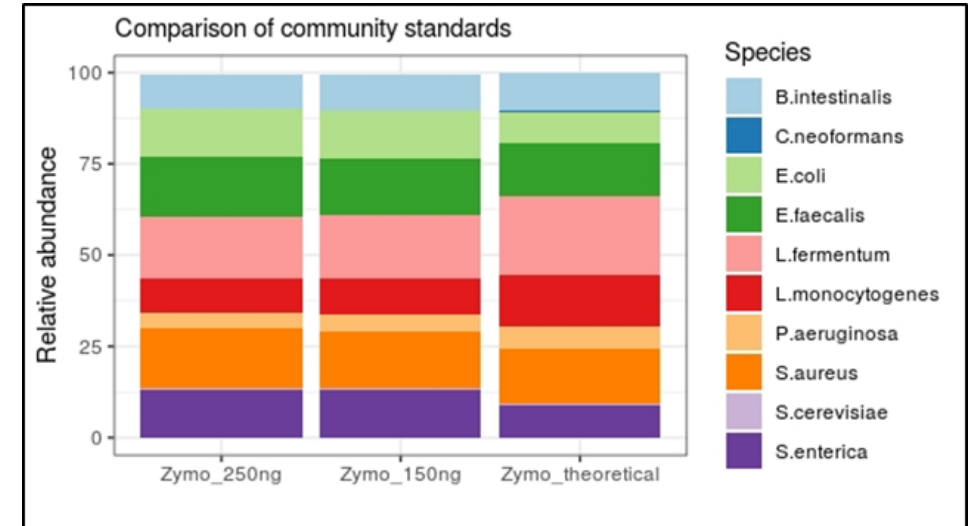
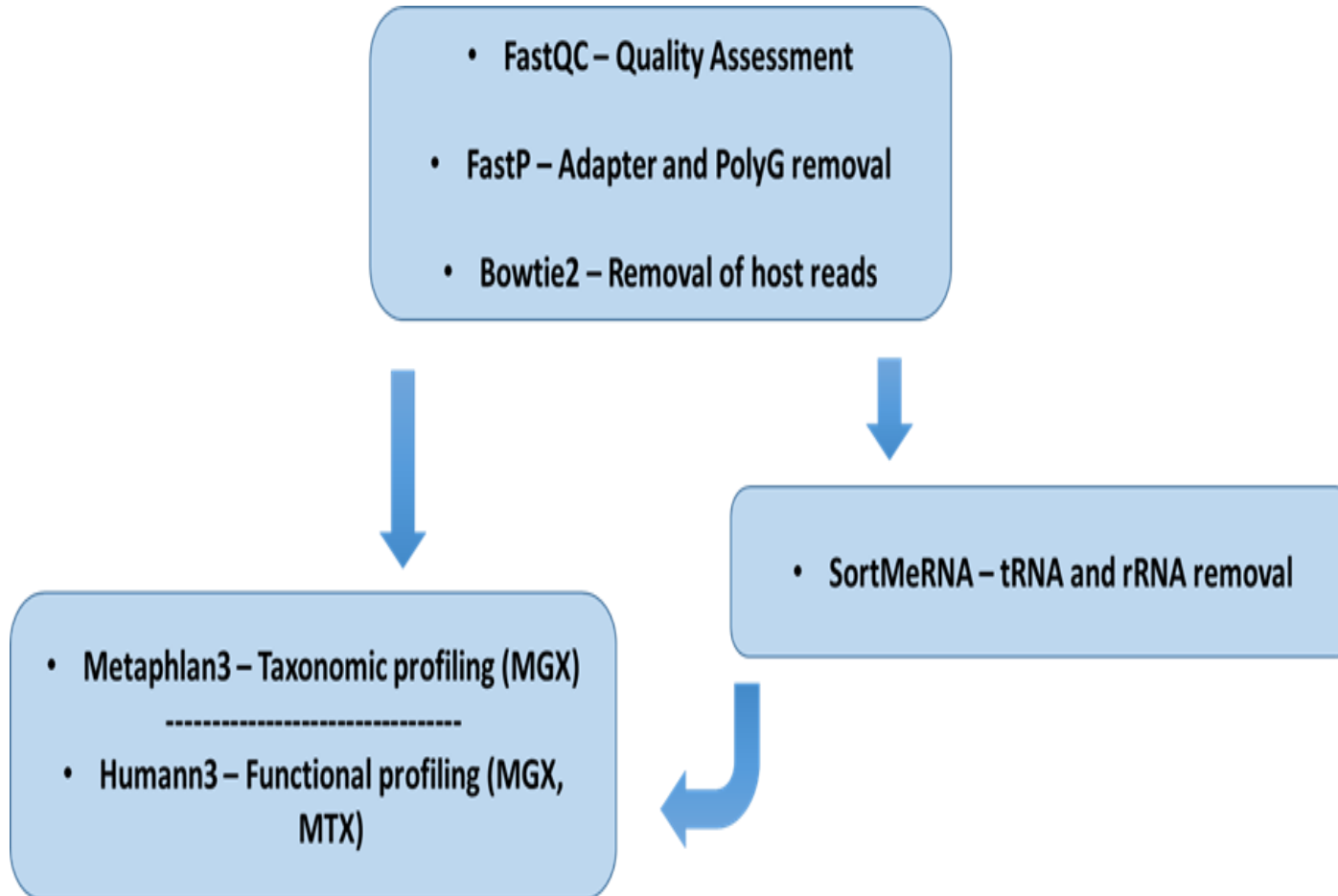
- Rumen fluid samples (20ml) obtained from 90 sheep
- Microbial DNA extracted using the Qiagen Powersoil Pro kit
 - DNA QC assessed on nanodrop and 0.8% agarose gel
 - Concentration assessed using Qubit
- 16S amplicon sequencing (n=90)
 - PCR amplification of V4 region 16S rRNA gene (Caporaso et al., 2011)
 - Illumina MiSeq 100,000 PE reads/sample
- Metagenomic/Metatranscriptomics sequencing
 - DNA: Illumina Novaseq 100m PE reads/sample
 - RNA: Illumina Novaseq 50m PE reads/sample



Metataxonomic analysis (16S)



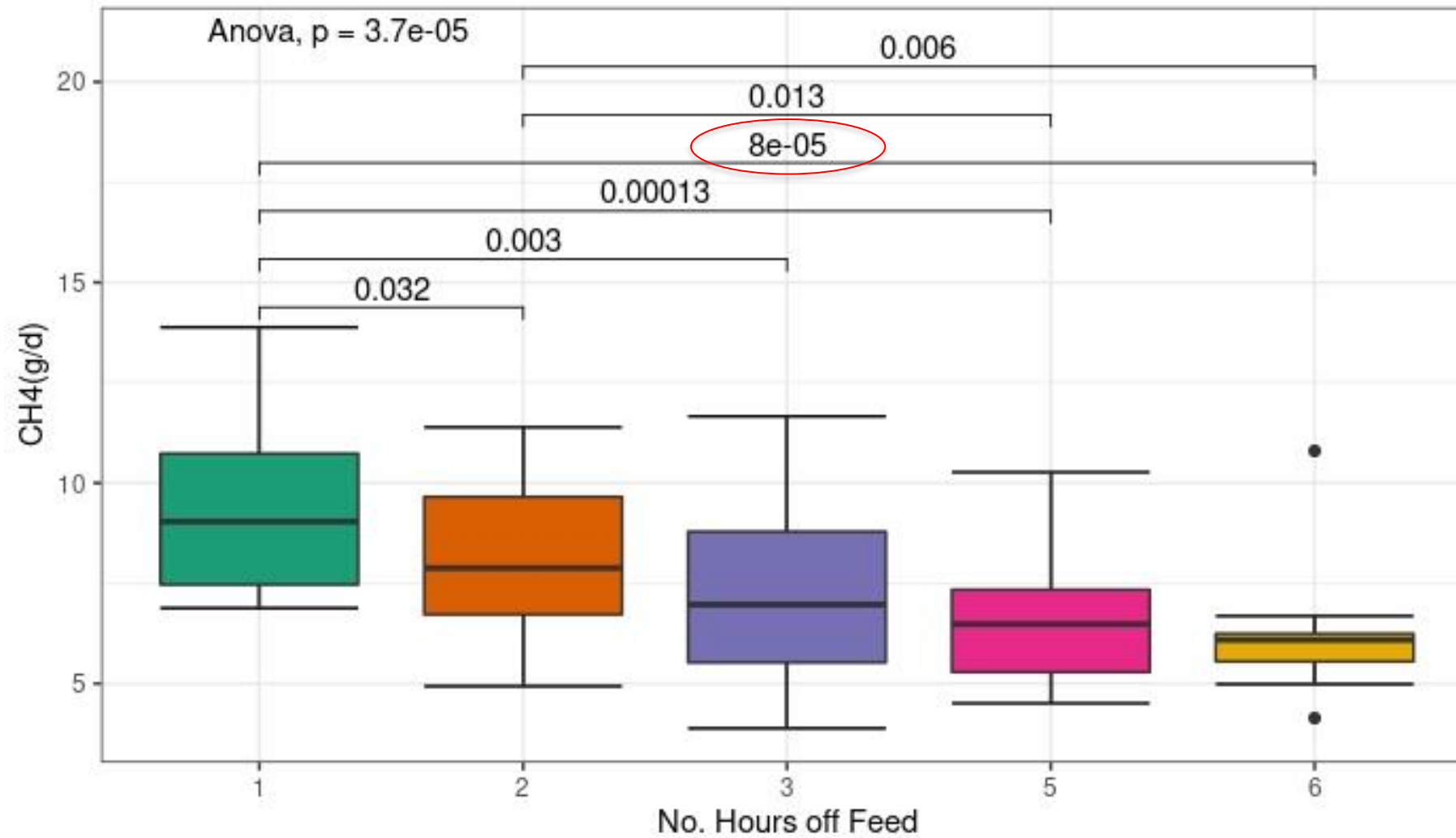
Meta-genomic/transcriptomic pipeline and analysis



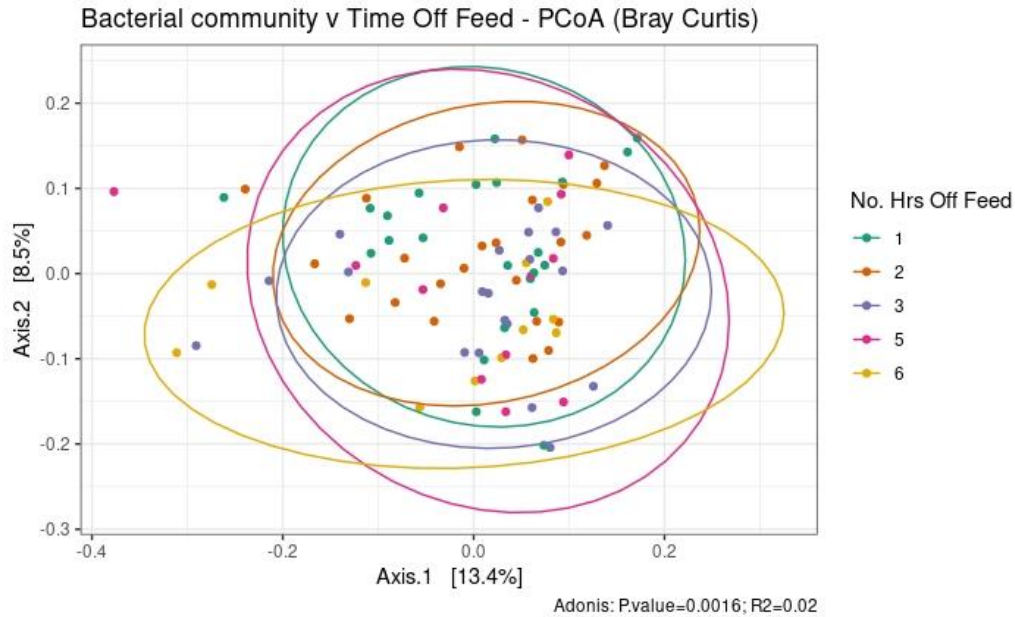
Metaphlan3 findings comparable with Zymo theoretical standards

Results

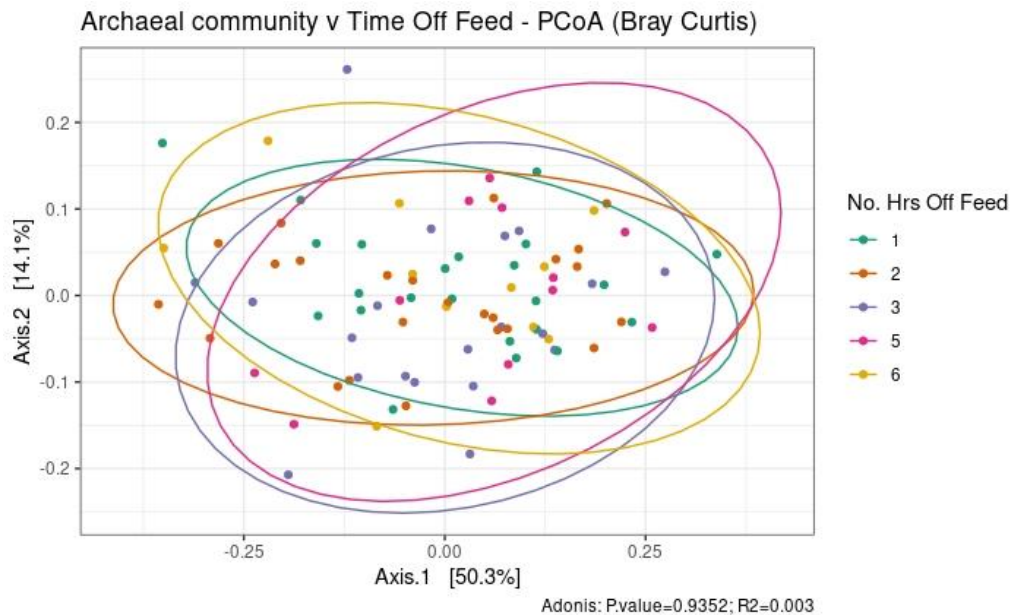
Effect of TOF on CH₄ emissions



Effect of TOF on microbial diversity

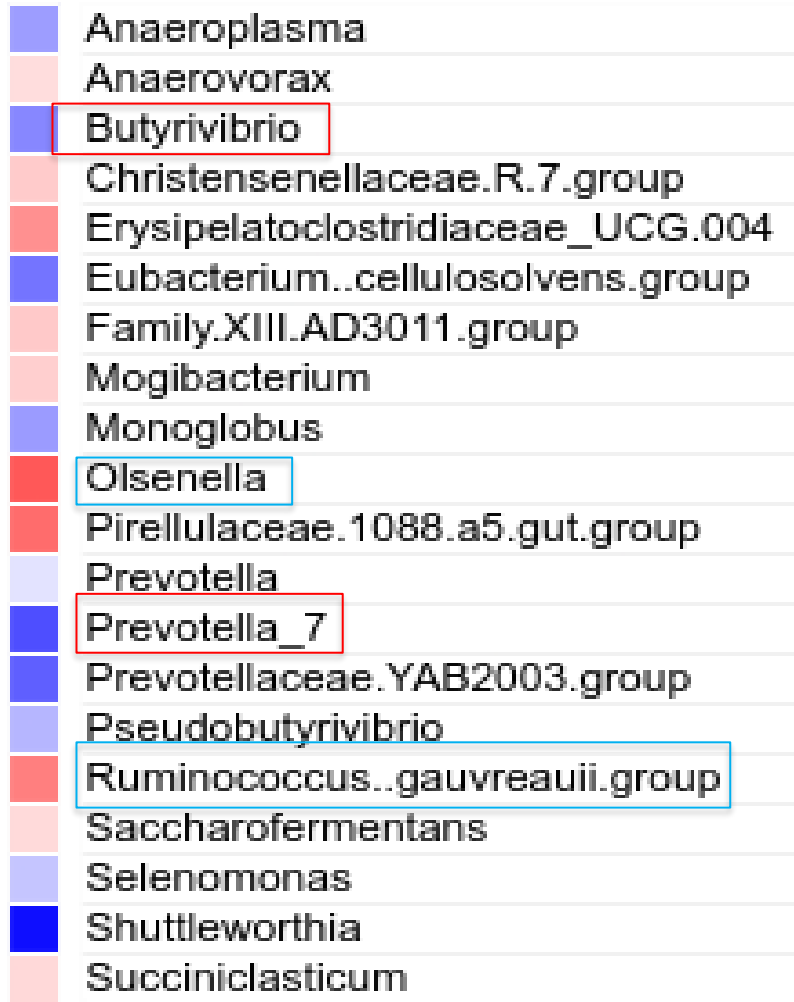


- Bacterial community composition significantly influenced by TOF ($P < 0.01$).
 - ~ 2% of the variance in community composition

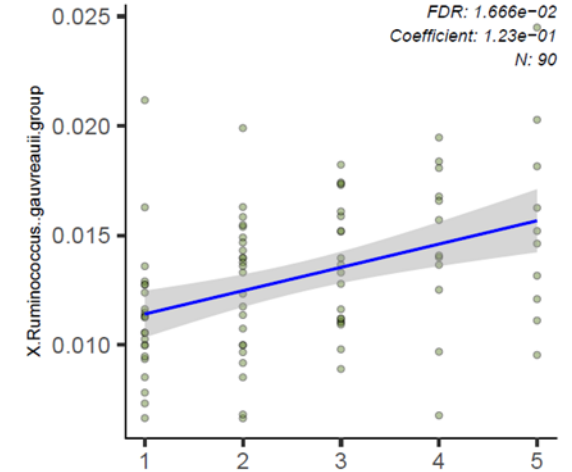
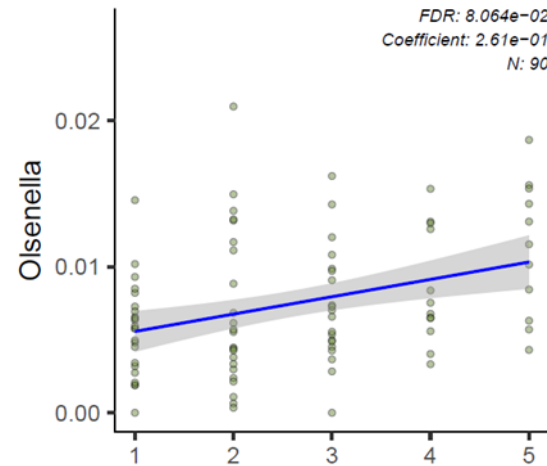
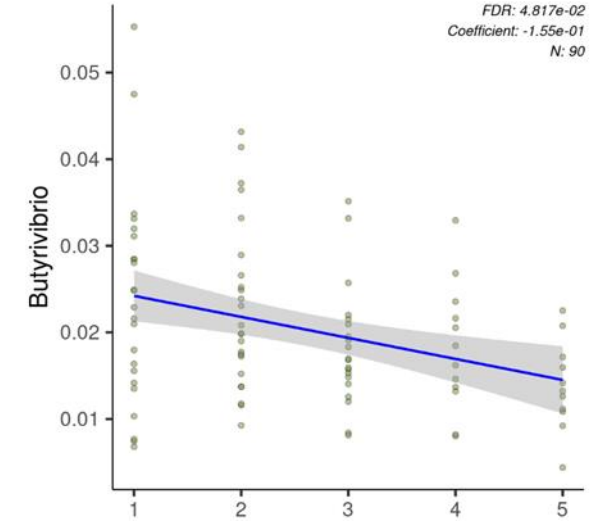
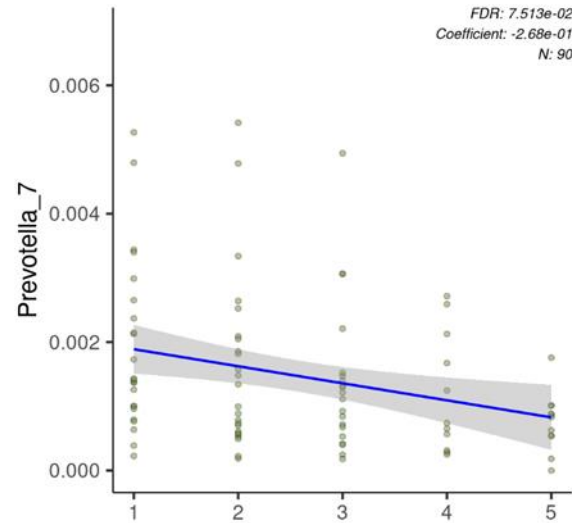


- No significant effect of TOF on Archaeal community ($P > 0.10$)

Bacterial genera associations with TOF



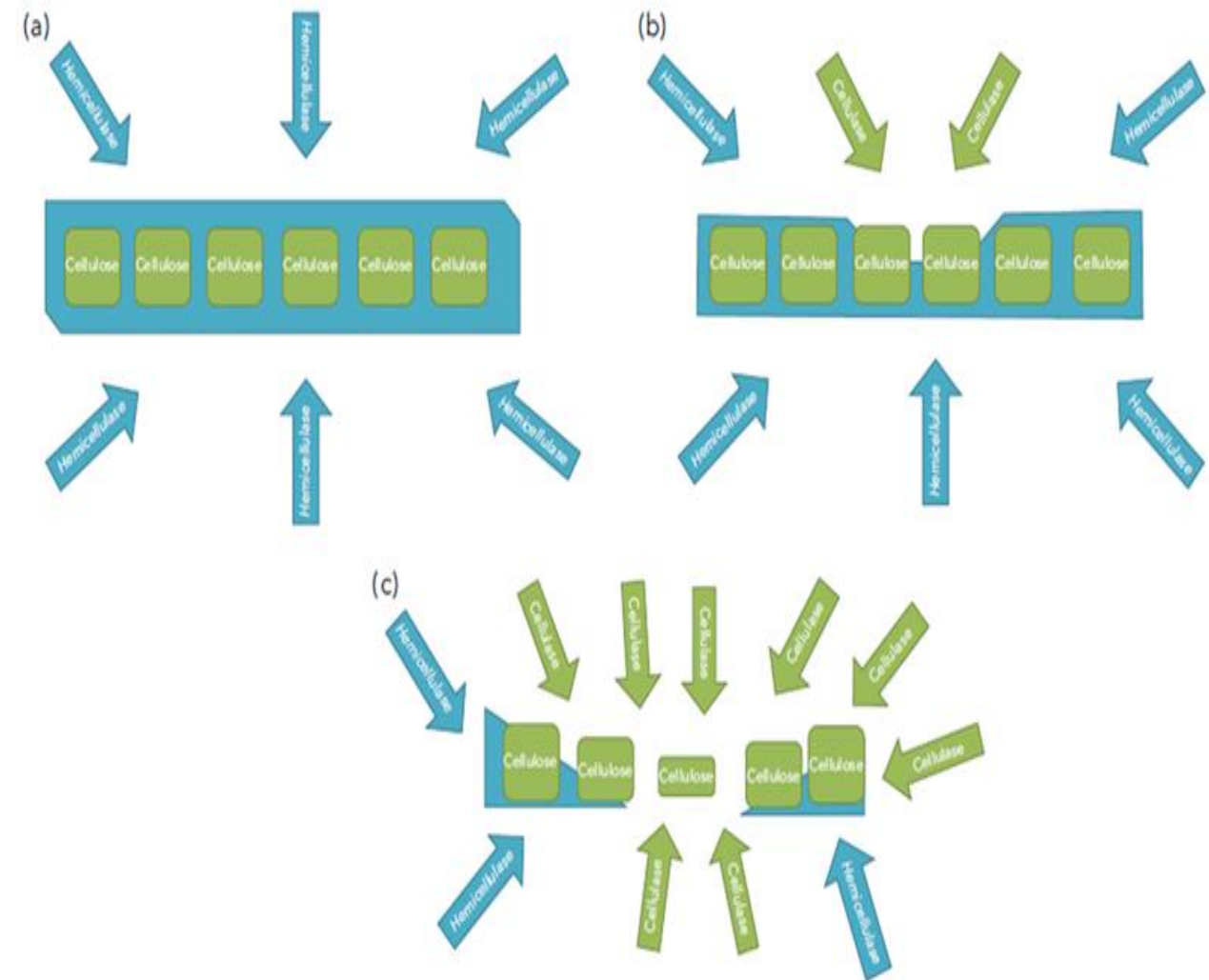
$(-\text{Log}(q\text{val}) \cdot \text{sign}(\text{coeff}))$



*1=1hr off feed, 2=2hrs off feed, 3 =3hrs off feed,
4=5hrs off feed, 5=6hrs off feed

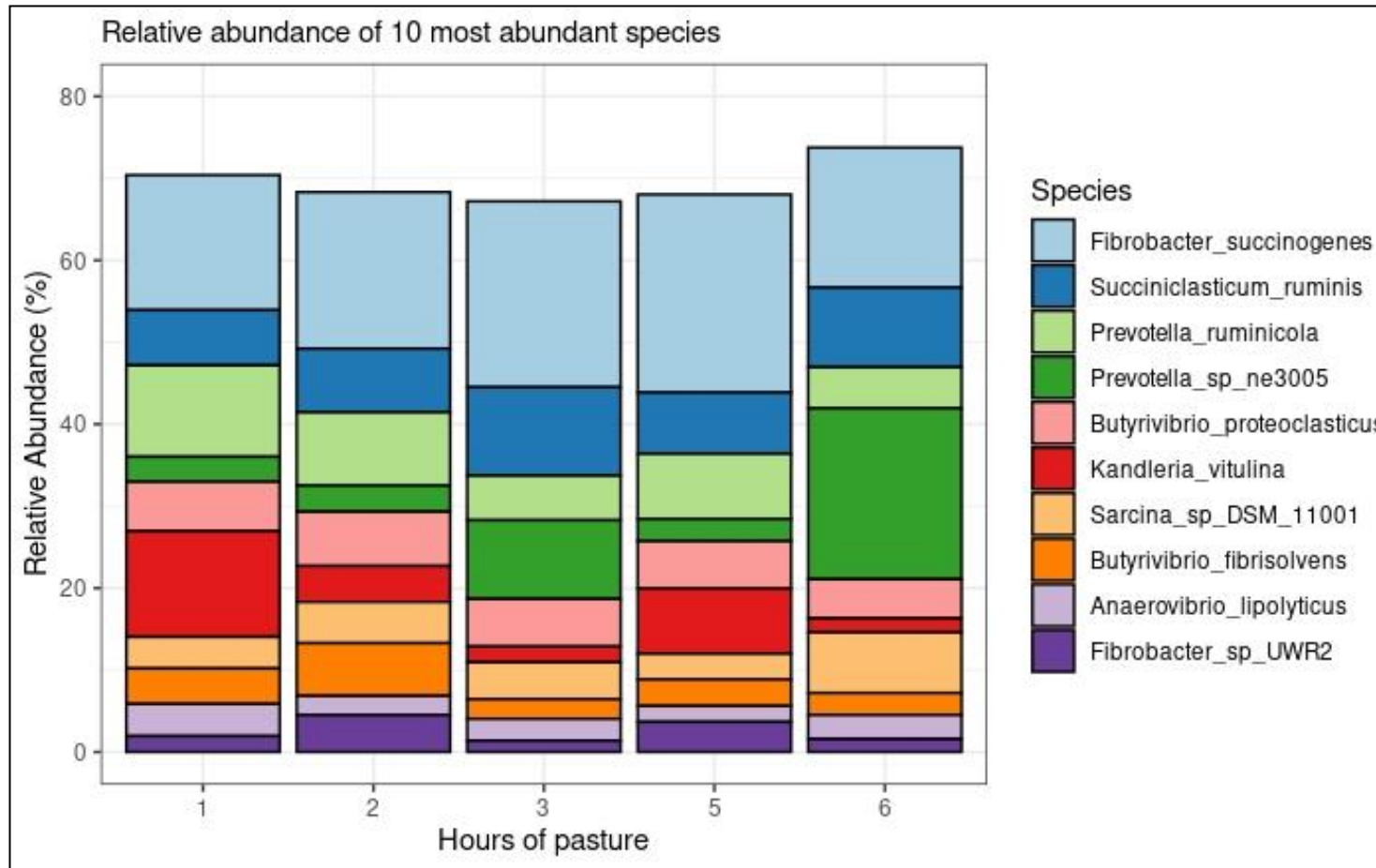
Putative mechanism of action

- Decrease in hemicellulolytic bacteria overtime
 - Major butyrate producers
 - Reduced production of hydrogen, and consequently methane with time
- Growth of microorganisms linked with non-structural carbohydrate metabolism



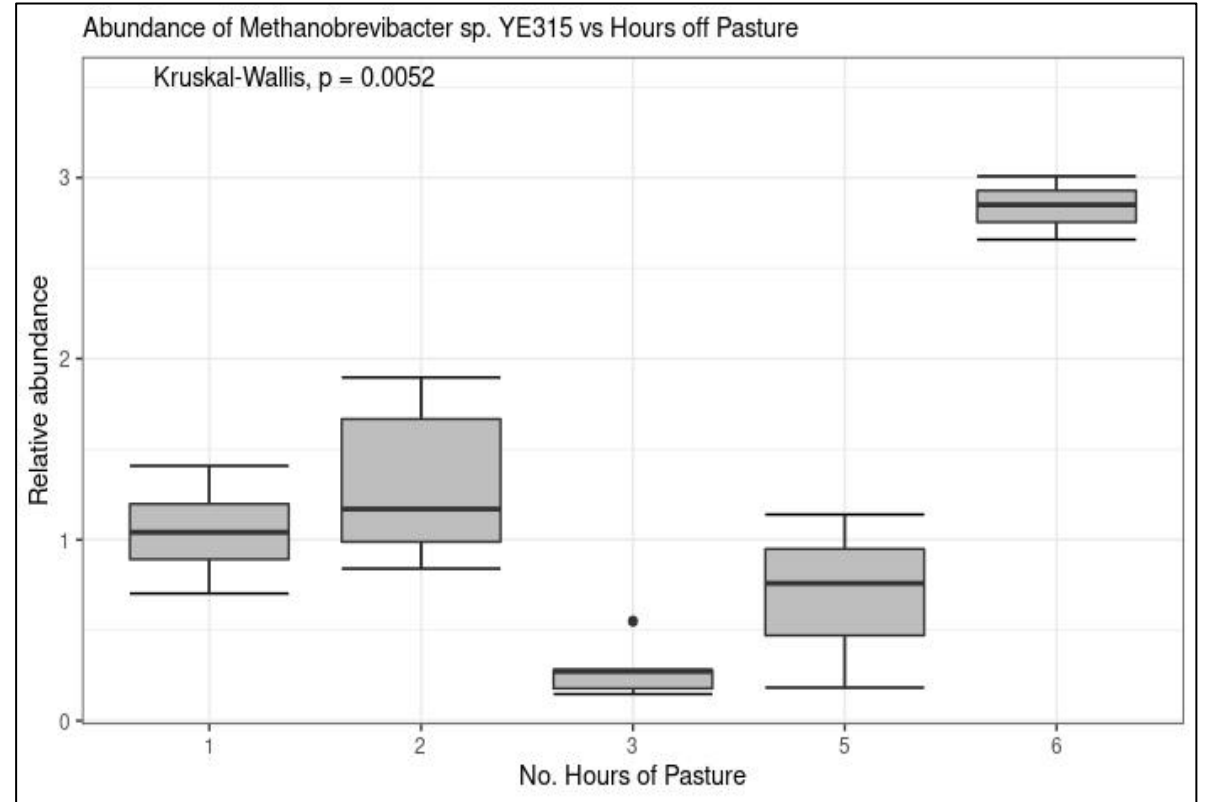
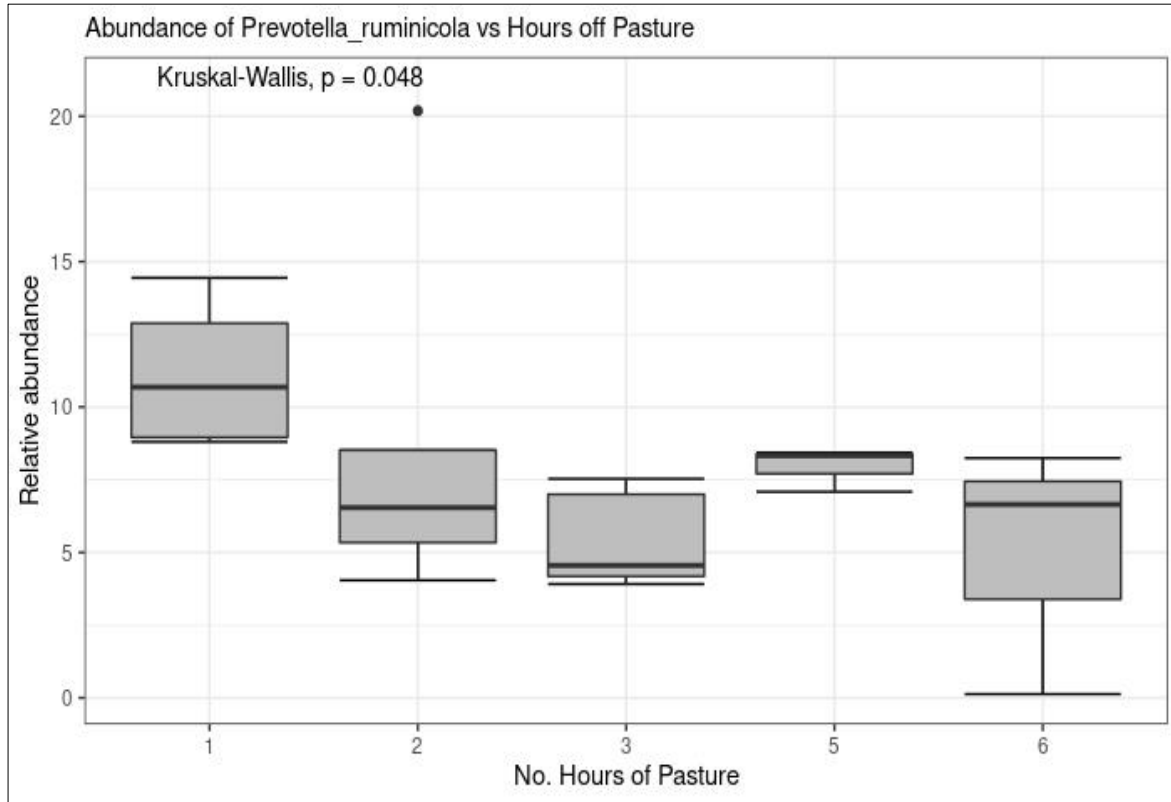
Exploratory analysis of meta-genome and meta-transcriptome data

Meta-genomic taxonomic profiling



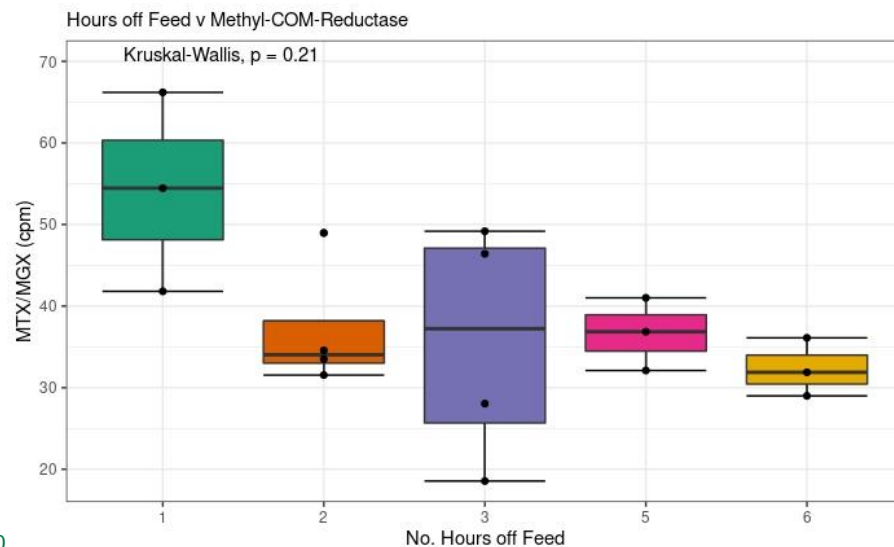
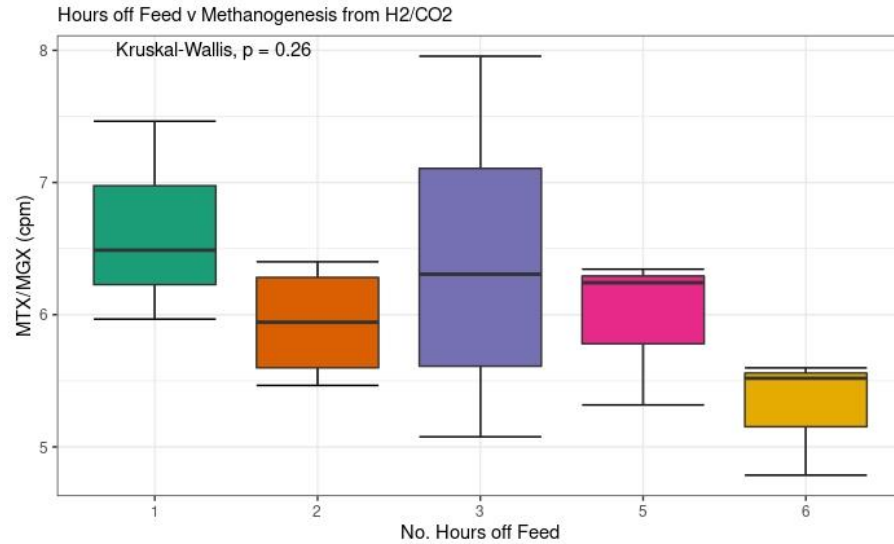
- Metaphlan identified 190 microbial species
- *Fibrobacter succinogenes*, *Succiniclasticum ruminis* and *Prevotella ruminicola* identified as the most abundant microbial species

Meta-genomic differential abundance



Effect of TOF on methanogenesis pathway and *mcrA* gene activity

- **Activity** of the methanogenesis pathway and the **transcription rate** of the *mcrA* gene have a tendency to decline over time



Summary

- TOF has been shown to have a significant influence on:
 - CH₄ emissions
 - Composition of the rumen bacterial community
- Based on our exploratory analysis, the activity of the methanogenesis pathway tended to decrease with increased TOF
- Implications for animal based research focused on the rumen microbiome and methane emissions

Thank you for your attention



Funding: EU Horizon: MASTER (Contract no. 818368)



Additional slides

DNA extraction and sequencing

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 - Illumina MiSeq
 - 100,000 PE reads per sample (except for neg control)
- Metagenomic sequencing (n=20)
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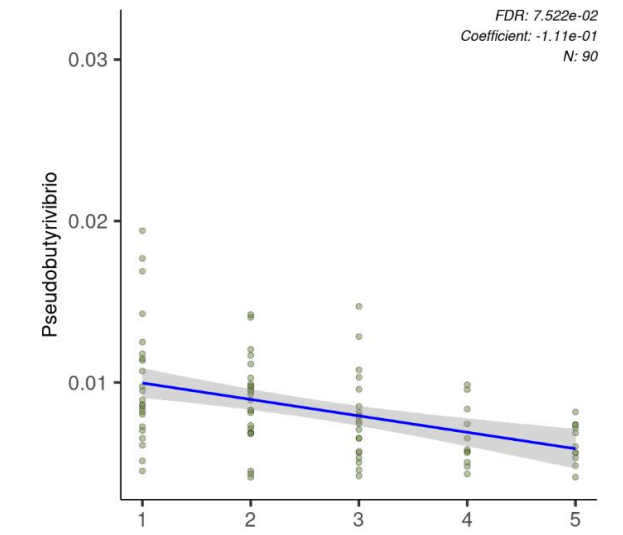
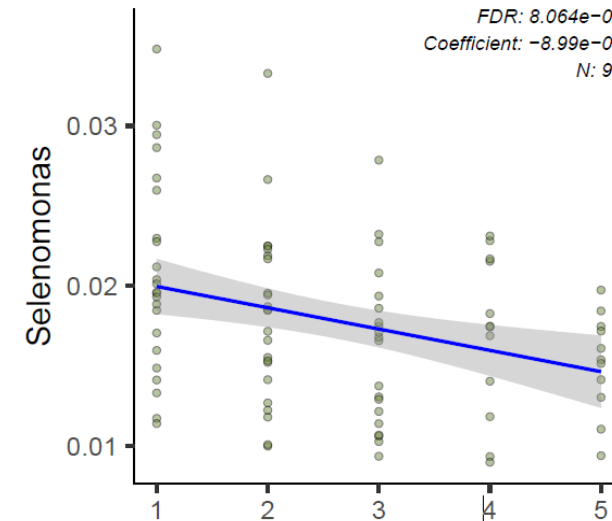
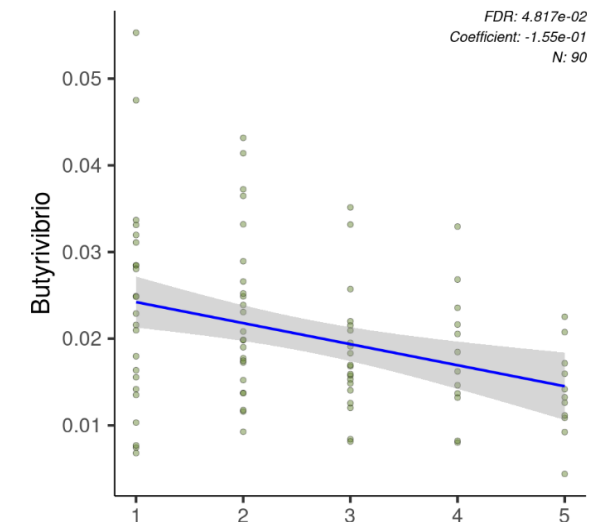
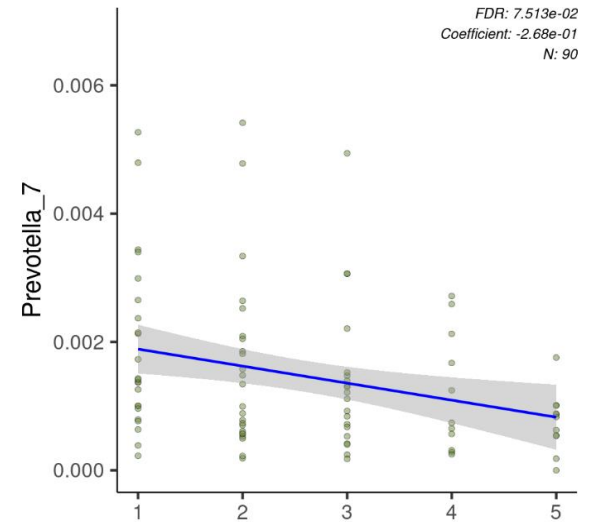
RNA extraction and sequencing

- Microbial RNA extracted Qiagen RNeasy plus kit (n=20)
 - Same samples utilised in metagenomics analysis
 - 200mg of ground rumen digesta sample
- Additional DNase treatment and clean up
 - TURBO DNA-free™ Kit
 - Zymo RNA clean and concentrate kit
- RNA quality assessed on Bioanalyzer
 - All samples RIN >7.0
- RNA sequenced on Illumina Novaseq
 - 50million PE reads per sample (except for neg control)



Influence of Time Off Feed on rel. abundance bacterial Genera

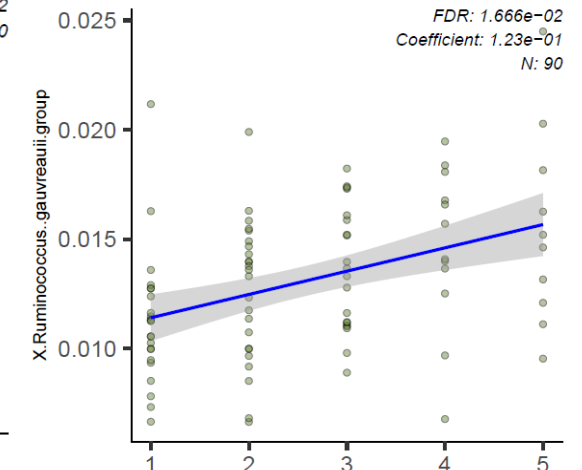
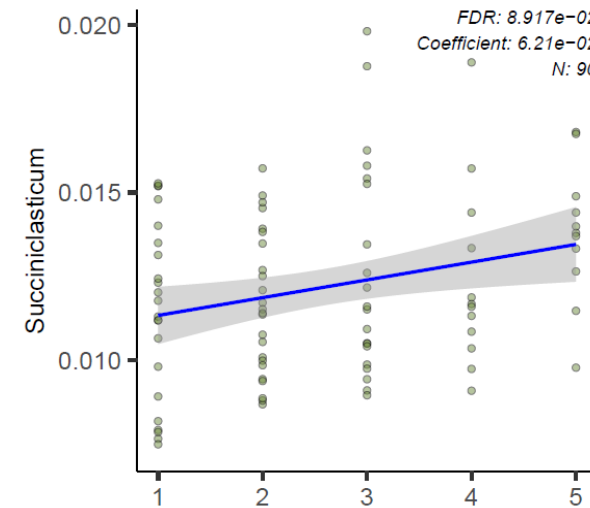
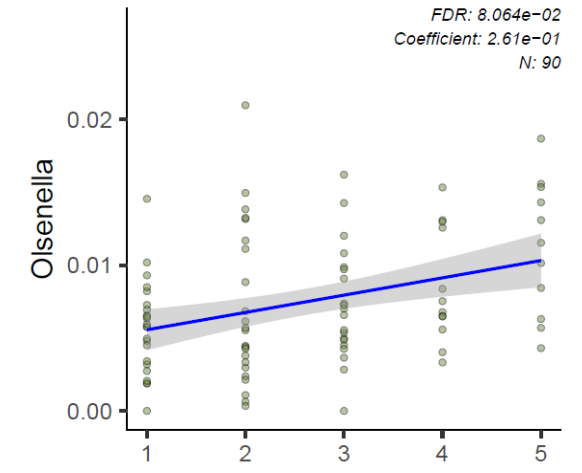
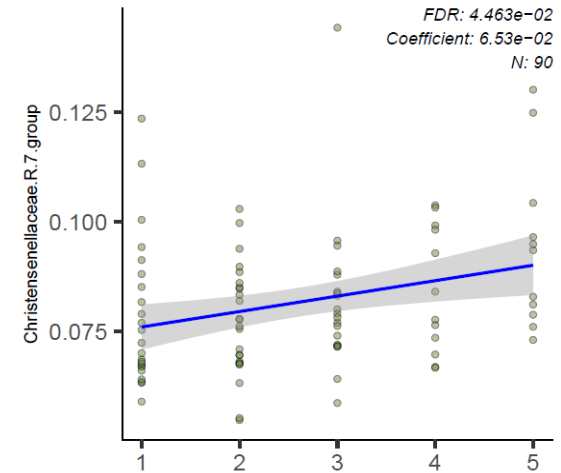
- Relative abundance of *Prevotella 7*, *Butyrvibrio*, *Pseudobutyvibrio*, and *Selenomonas* tend to decrease over time



Time off feed

Influence of Time Off Feed on Rel. abundance bacterial Genera

- Abundance of *Olsenella*, *Ruminococcus*, and *Christensenellaceae R7* group and *Succiniclasticum* tend to become more abundant with time

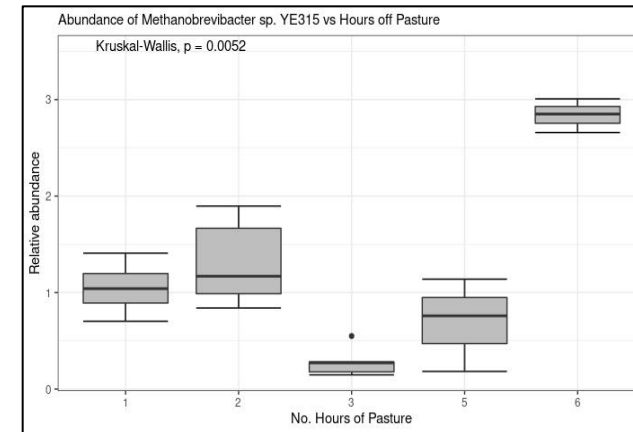
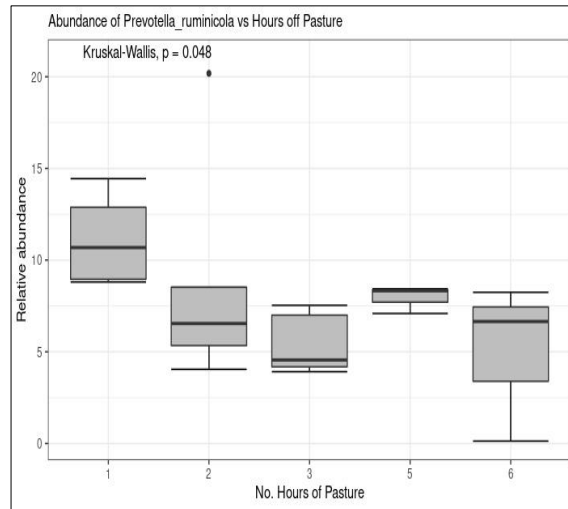


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