

Host effects on rumen microbiome – lessons from reindeer and twin cow studies

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Rationale

- Studies in mice and men indicate host genetic effect on the gut microbiome
- How about the rumen microbiome?



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Host specificity of the ruminal bacterial community in the dairy cow following near-total exchange of ruminal contents¹

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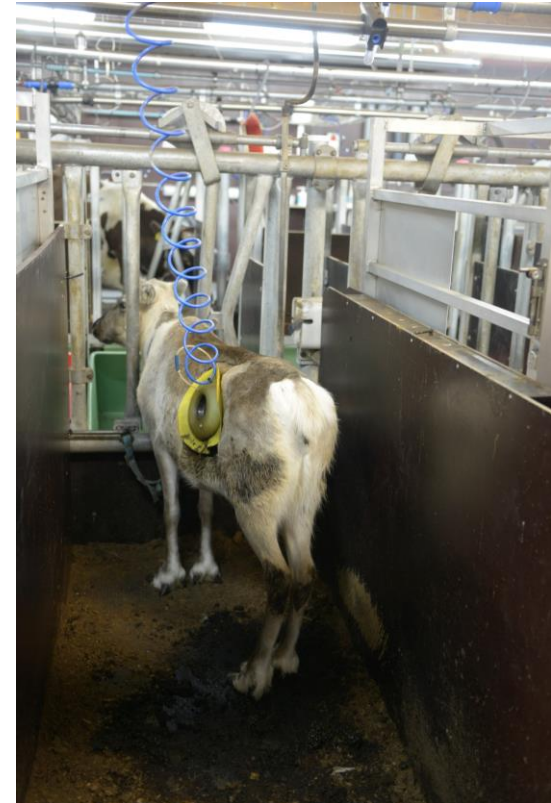
- Ruminal bacterial community composition displays host specificity that **can re-establish itself with varying success** when challenged with a microbial community adapted to of a different host

Hypotheses

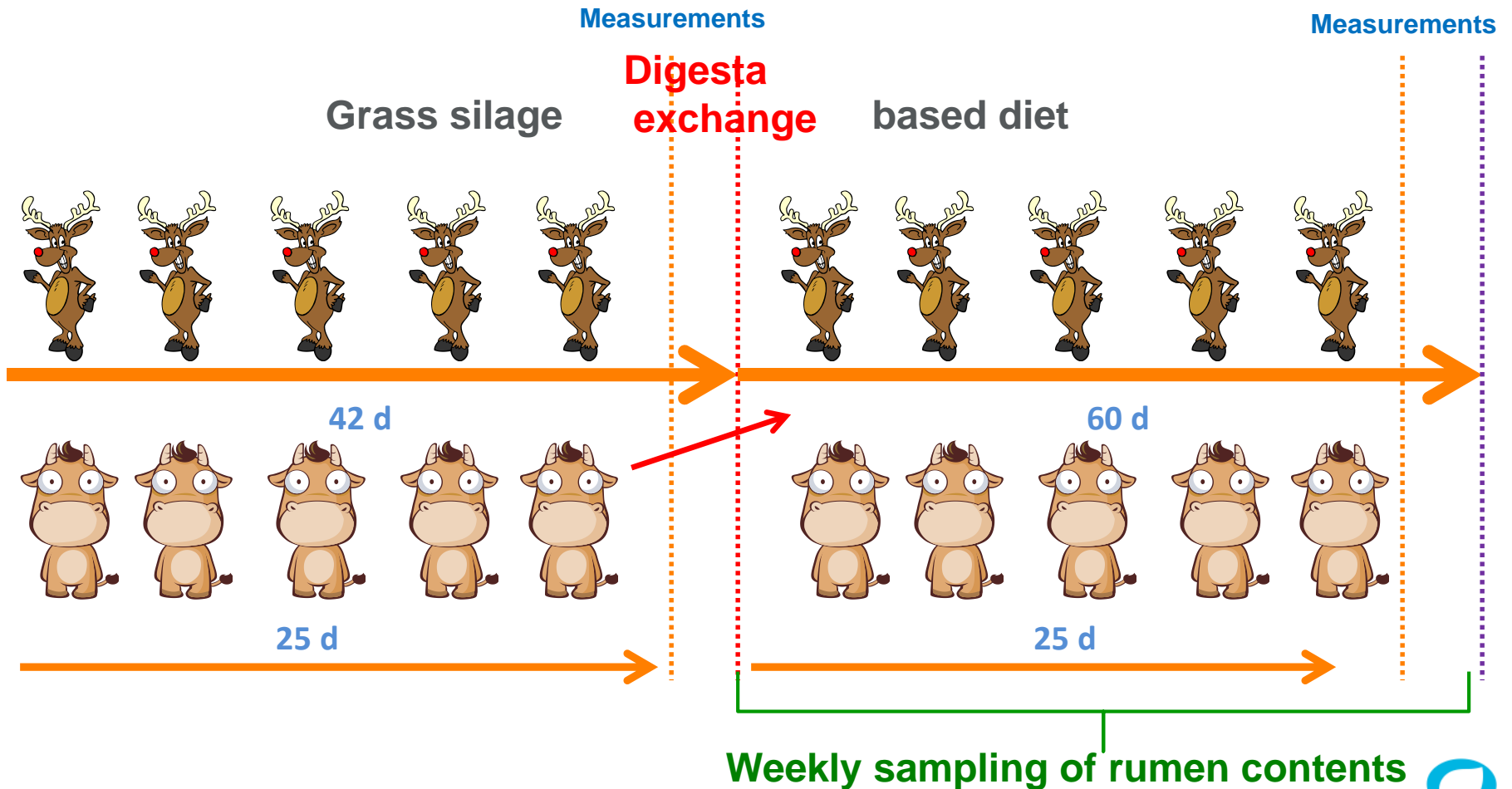
- **The host animal controls its own microbiome which influences rumen function, enteric methane production and nutrient digestion**
- **Differences in the rumen microbiome composition are smaller between genetically similar animals compared with genetically more distant animals**
- **If rumen contents are exchanged between genetically different animals, the rumen microbiome composition will revert towards original composition of the host**

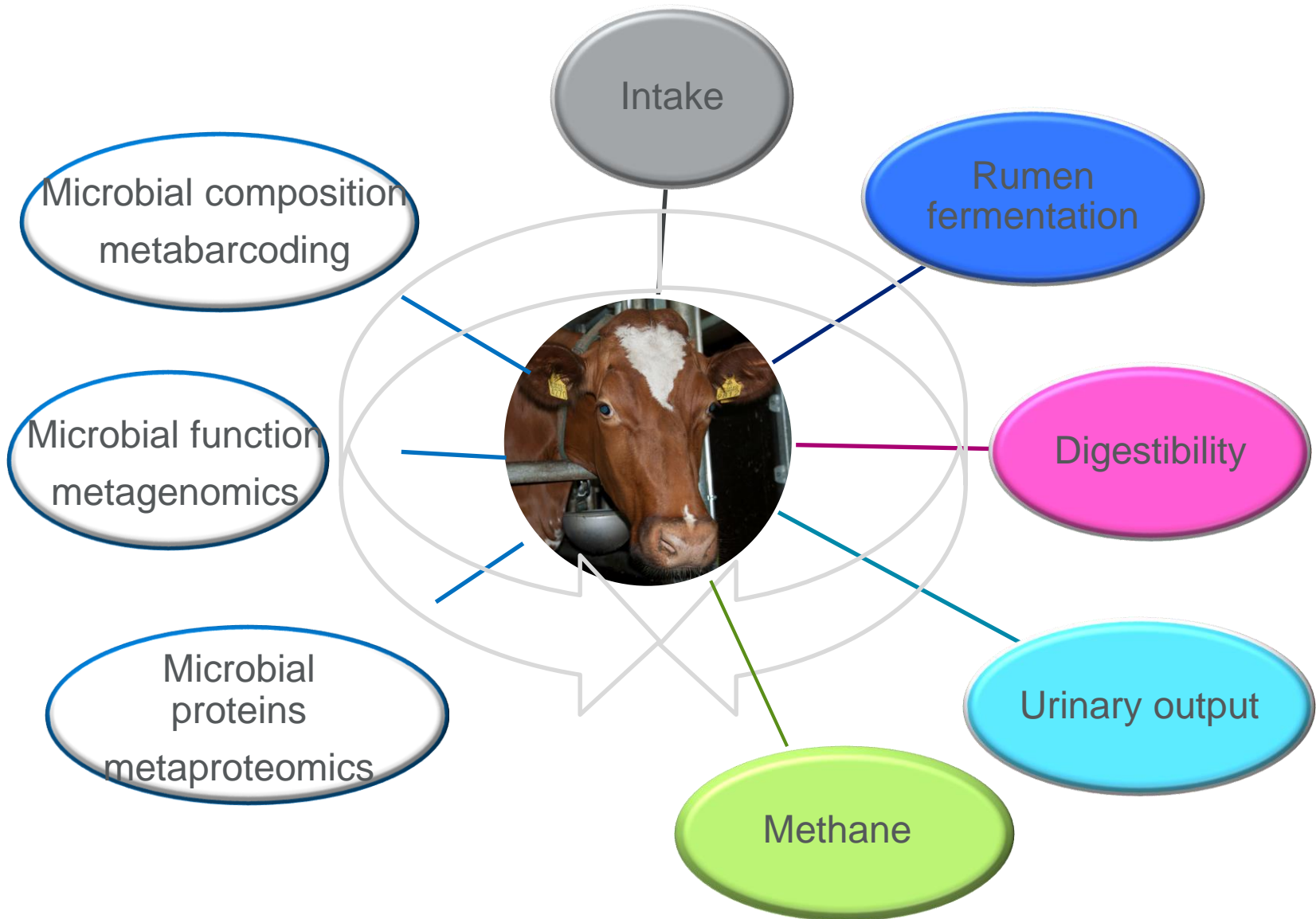
Experiments

- Total exchange of rumen contents
- Between cows and reindeer
- Between identical twin cows and unrelated cows



Reindeer experimental setup

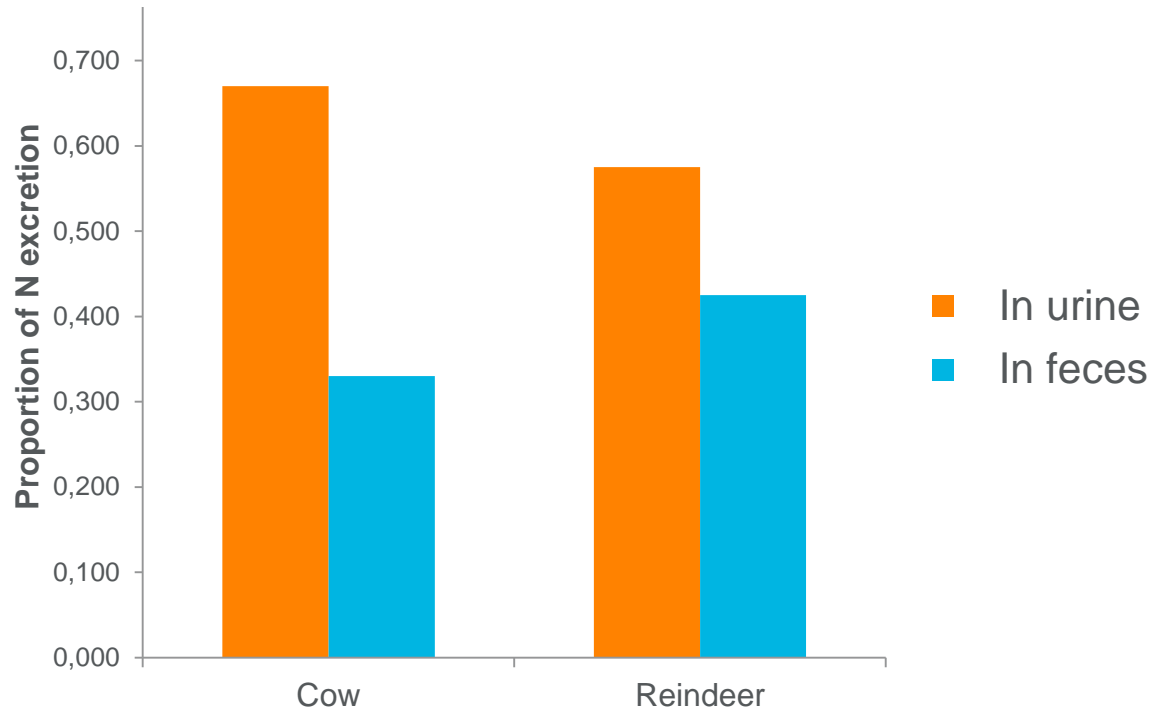




Intake, digestibility and methanogenesis

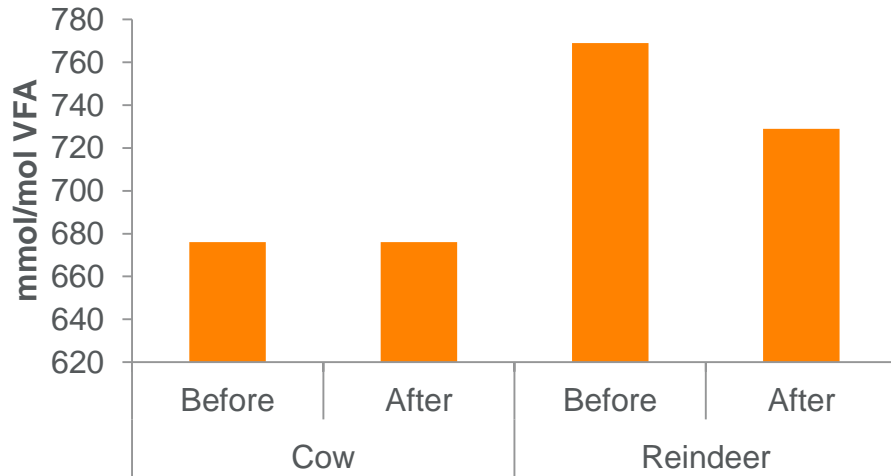
| | Cow | | Reindeer | | SE | P values | | |
|----------------------|-------|-------|----------|-------|--------|----------|-------|----------|
| | Pre- | Post- | Pre- | Post- | | Sp | Trt | Sp × Trt |
| Intake, kg/d | | | | | | | | |
| DM, kg/d | 8.02 | 8.41 | 1.33 | 1.54 | 0.202 | <0.01 | <0.01 | <0.01 |
| DM, g/kg LW | 11.0 | 11.4 | 16.9 | 19.3 | 0.36 | <0.01 | <0.01 | <0.01 |
| Digestibility | | | | | | | | |
| OM, g/g | 0.722 | 0.745 | 0.728 | 0.741 | 0.0073 | 0.95 | <0.01 | 0.36 |
| NDF, g/g | 0.634 | 0.650 | 0.639 | 0.658 | 0.0108 | 0.60 | 0.04 | 0.88 |
| Methane | | | | | | | | |
| g/d | 198 | 184 | 23.7 | 30.7 | 8.58 | <0.001 | 0.29 | <0.01 |
| mg/kg live weight | 271 | 249 | 302 | 384 | 23.0 | <0.01 | 0.09 | <0.05 |
| g/kg DMI | 24.6 | 21.9 | 17.8 | 19.9 | 1.34 | <0.01 | 0.75 | <0.05 |
| g/kg DOMI | 37.3 | 32.3 | 26.8 | 29.3 | 1.74 | <0.001 | 0.36 | <0.05 |

Nitrogen excretion

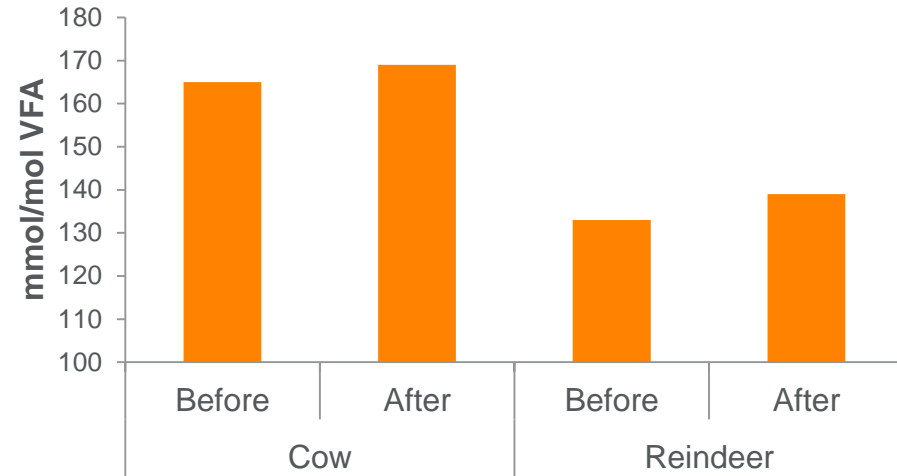


| | Cow | | Reindeer | |
|----------|--------|-------|----------|-------|
| | Before | After | Before | After |
| In urine | 0.66 | 0.68 | 0.59 | 0.56 |
| In feces | 0.34 | 0.32 | 0.41 | 0.44 |

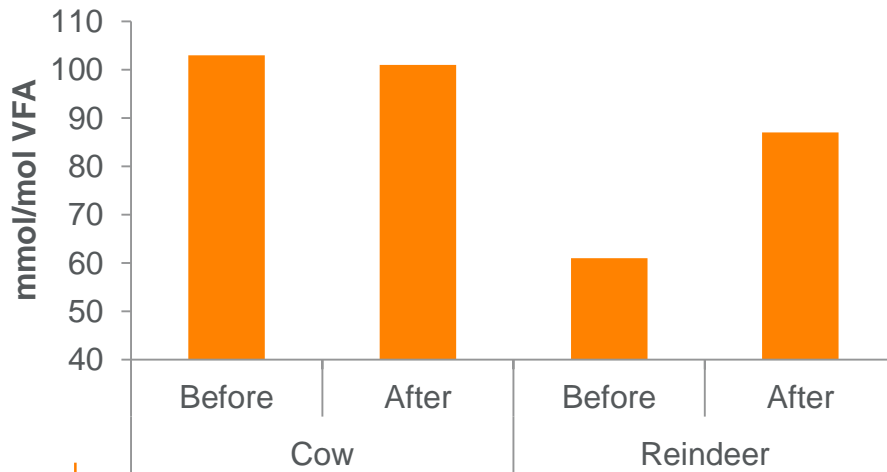
Acetate



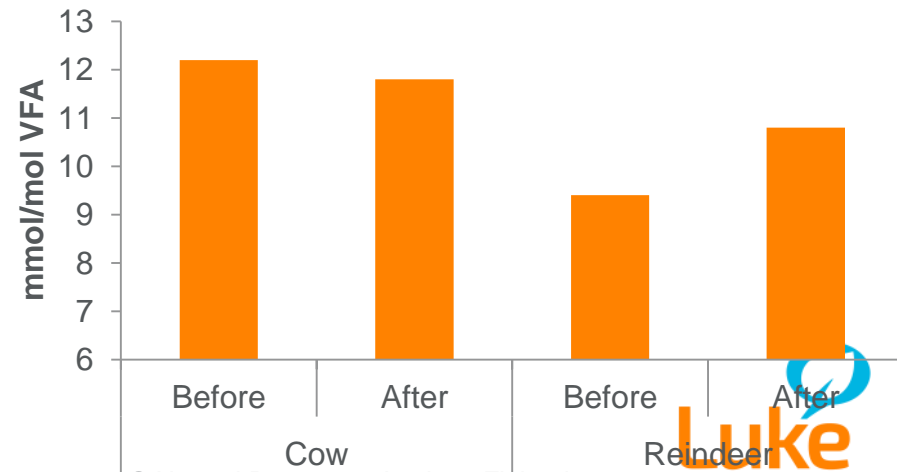
Propionate



Butyrate



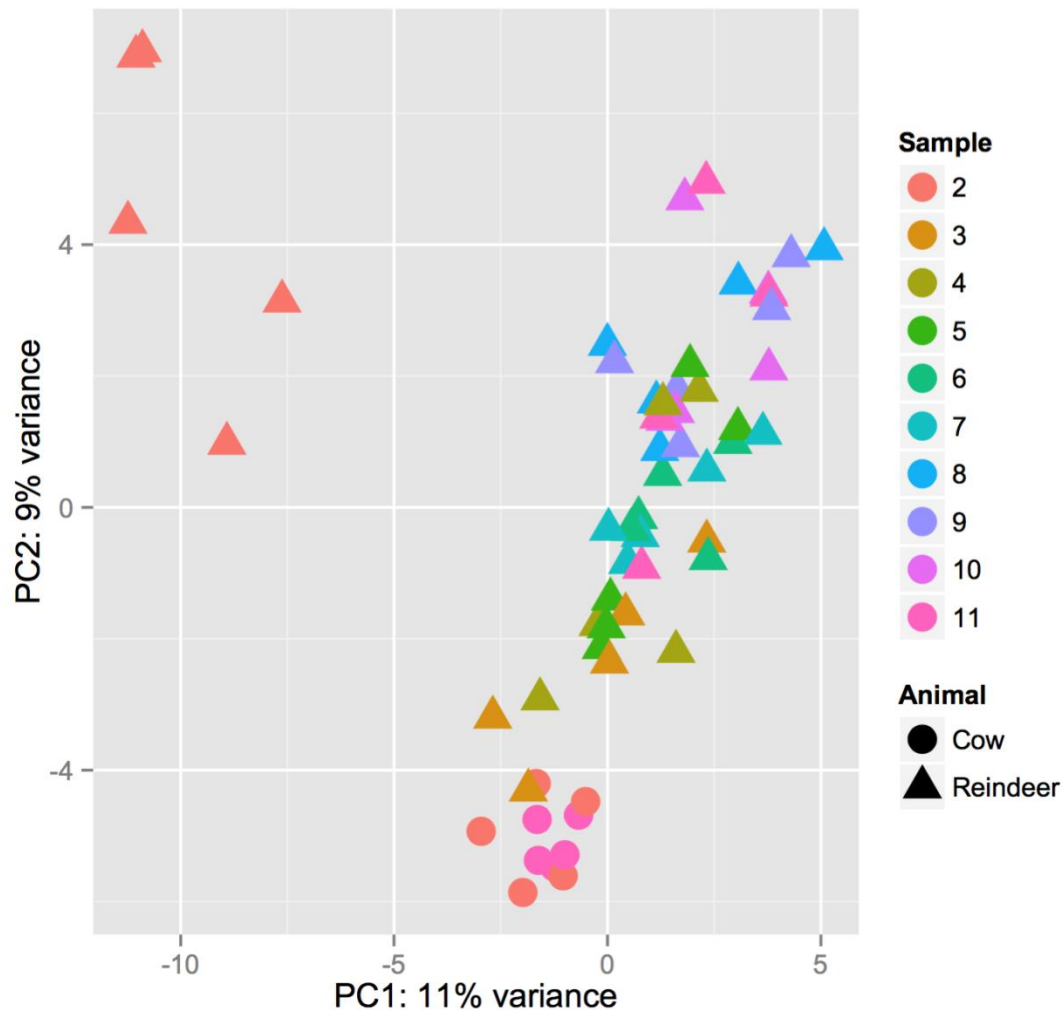
Isobutyrate



Emissions and ruminant species

- **Rumen fermentation characteristics differ between ruminant species**
- **Reindeer produce less methane per unit of digestible organic matter intake than cows**
- **Reindeer excrete a higher proportion of dietary nitrogen in faeces and less in urine compared with cows**

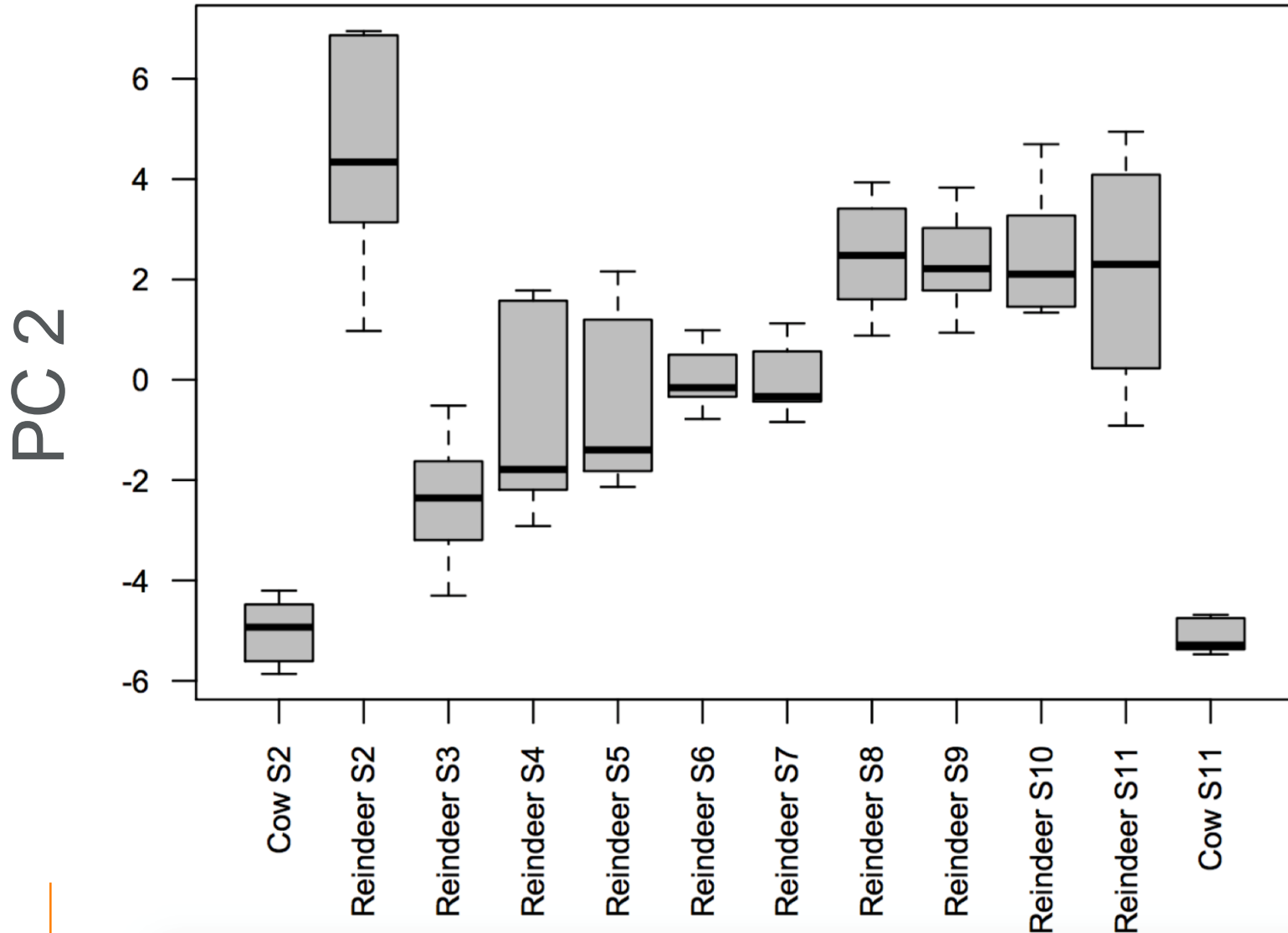
Influence of ruminant species on the rumen bacterial microbiome



The first PC: differences in rumen samples from cows (circles) and reindeer (triangles)

The second PC: the change over time in rumen samples collected from reindeer at weekly intervals

Trend observed over time in PC 2 towards the starting point

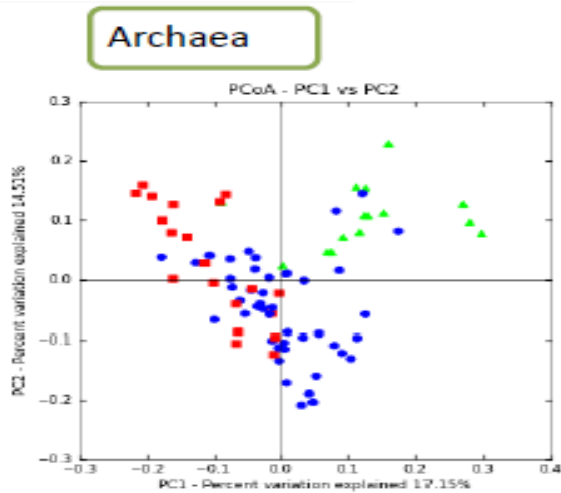


Principal coordinate analysis of microbial diversity Bray-Curtis dissimilarities matrix

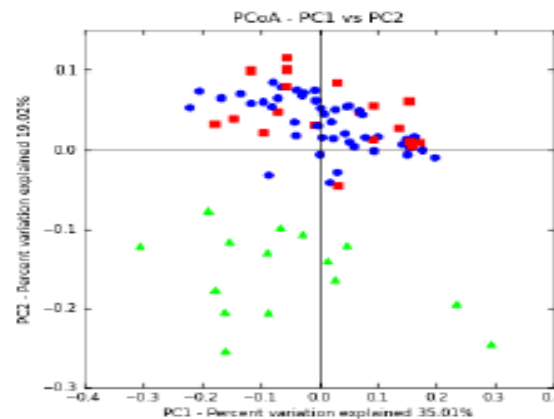
● Reindeer pre-exchange

● Reindeer post-exchange

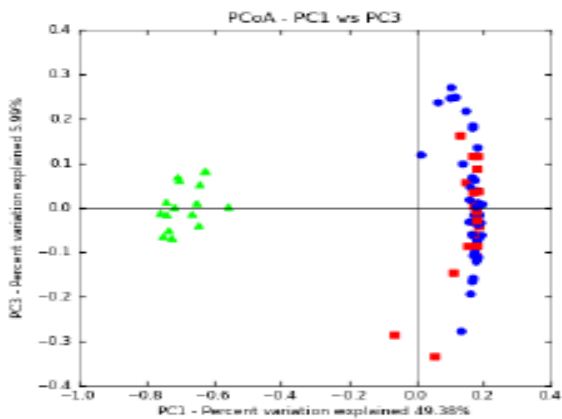
● Cows pre-exchange



Ciliate protozoa



Anaerobic fungi



Reindeer specific OTUs

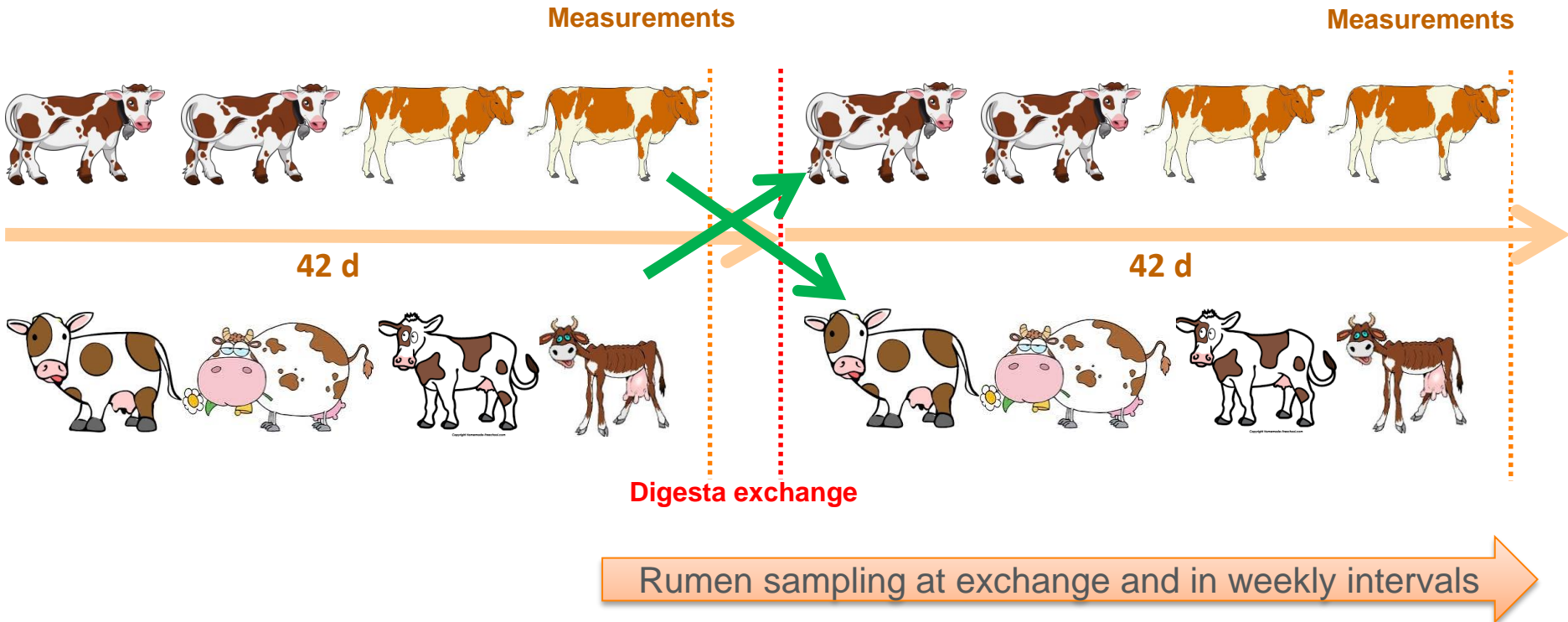
- 22 % of OTUs were specific to reindeer (13 % to cow)
- Reindeer OTUs could not be identified beyond phylum level, e.g.

| | |
|--------|---------------|
| OTU32 | Bacteroidetes |
| OTU295 | Bacteroidetes |
| OTU136 | Firmicutes |
| OTU166 | Firmicutes |
| OTU122 | Firmicutes |
| OTU429 | Bacteroidetes |
- metagenomic data will be used to explore these enigmatic species and why they may be associated with the reindeer

Host effects on rumen function and the microbiome

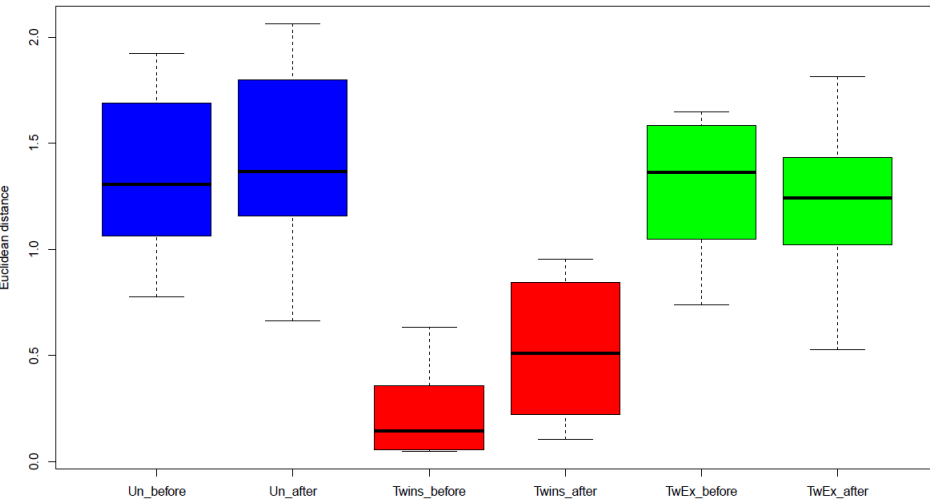
- A proportion of the microbial community in the rumen was specific to cows or reindeer
- A core microbiome was common to both species
- After digesta exchange microbial communities in the rumen of reindeer were more similar to that of cows than the original populations
- Evidence of changes in species specific to reindeer over time, consistent with an effect of the host animal

Twin cow experimental design



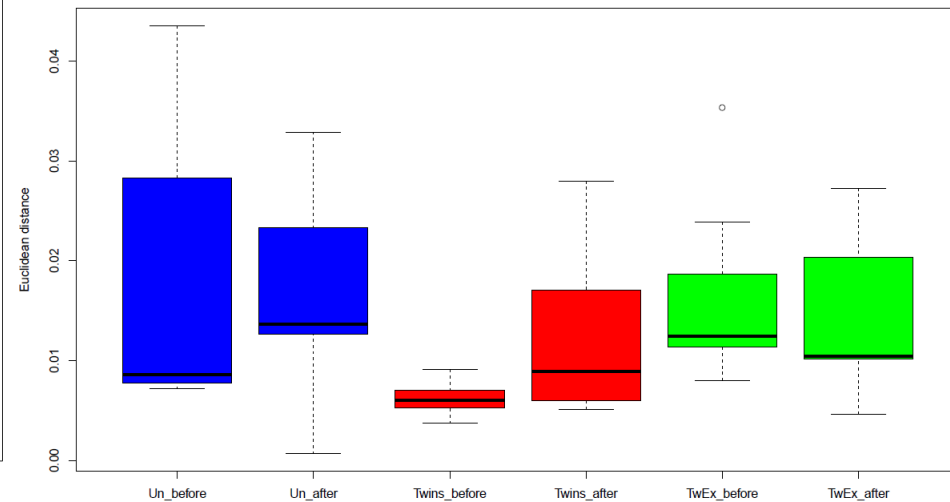
Phenotypic similarity

Milk yield



| | | | | | |
|---------------------|--------------------|-----------------|----------------|--------------------------|-------------------------|
| Unrelated Before | Unrelated After | Twins Before | Twins After | Twin-unrelated Before | Twin-unrelated After |
|---------------------|--------------------|-----------------|----------------|--------------------------|-------------------------|

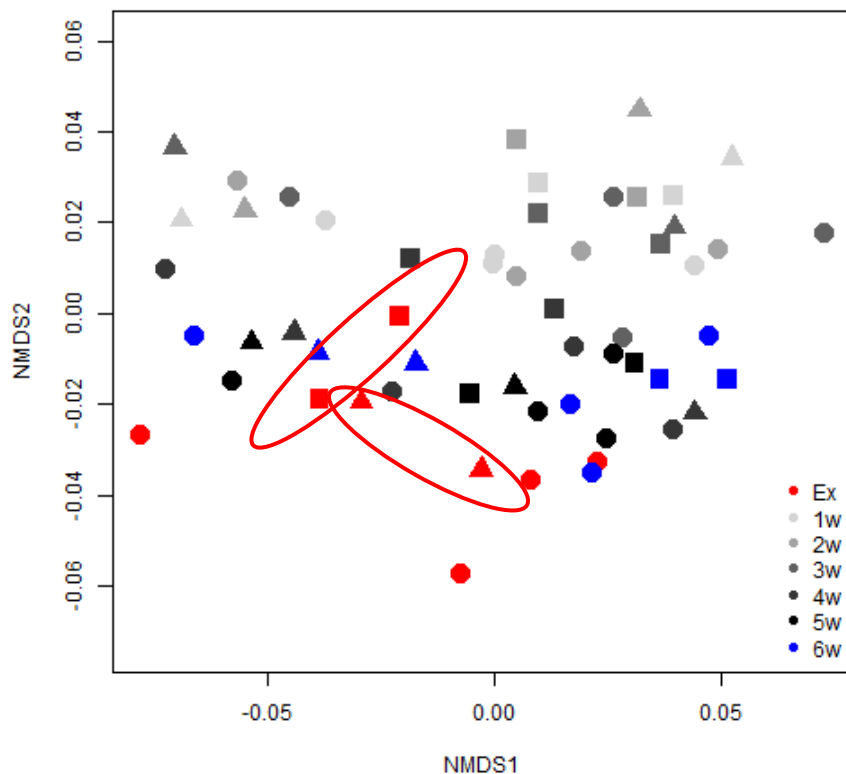
Digestibility



| | | | | | |
|---------------------|--------------------|-----------------|----------------|--------------------------|-------------------------|
| Unrelated Before | Unrelated After | Twins Before | Twins After | Twin-unrelated Before | Twin-unrelated After |
|---------------------|--------------------|-----------------|----------------|--------------------------|-------------------------|

Rumen microbiome

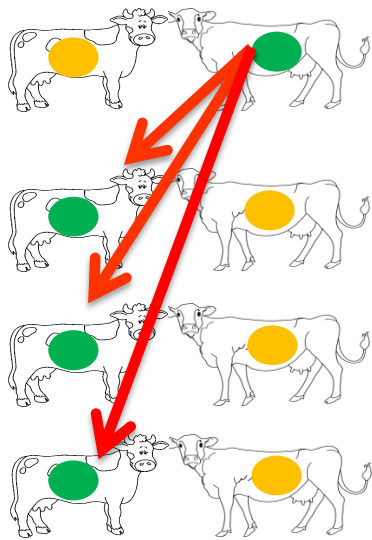
Bacteria family



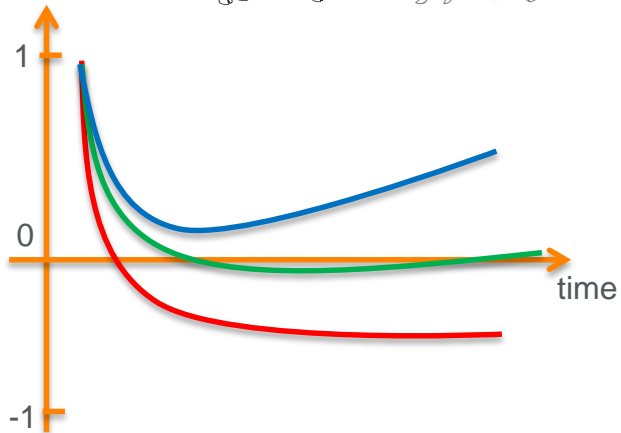
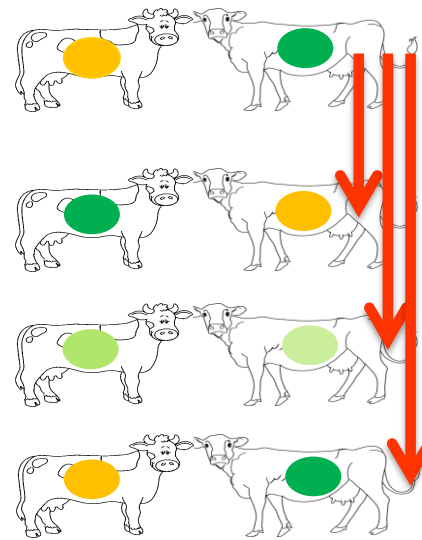
Red = before exchange
Blue = six weeks after exchange

Squares = twin pair 1
Triangles = twin pair 2
Dots = unrelated cows

Rumen-Rumen



Animal-Animal



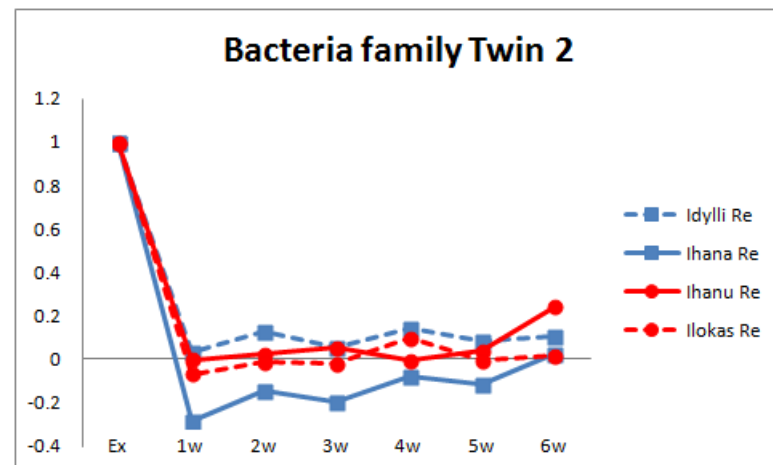
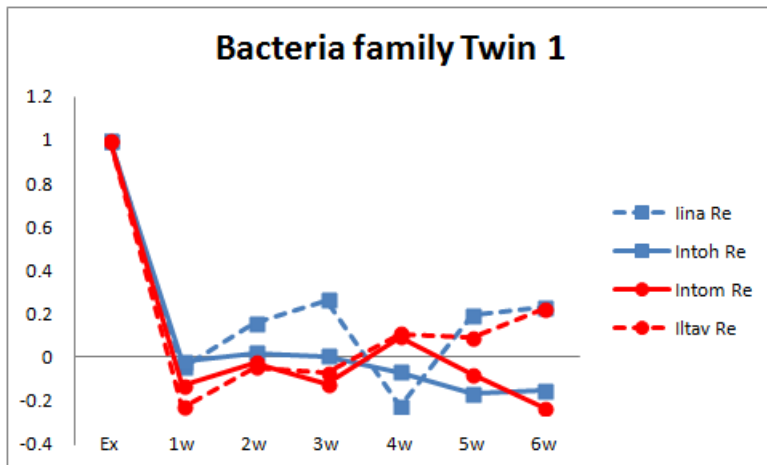
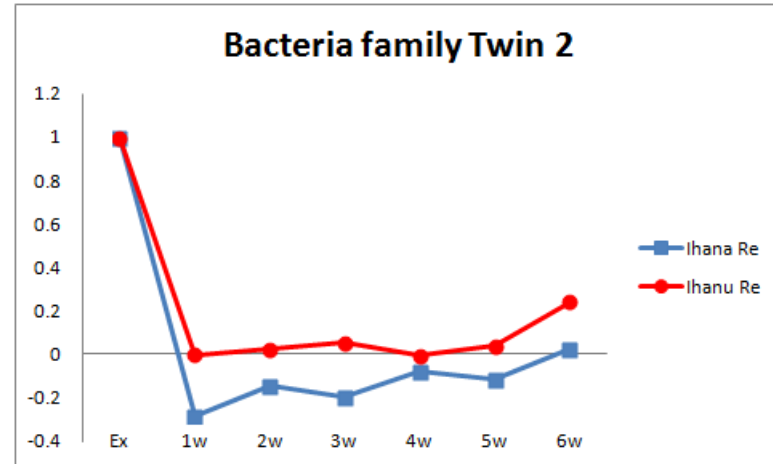
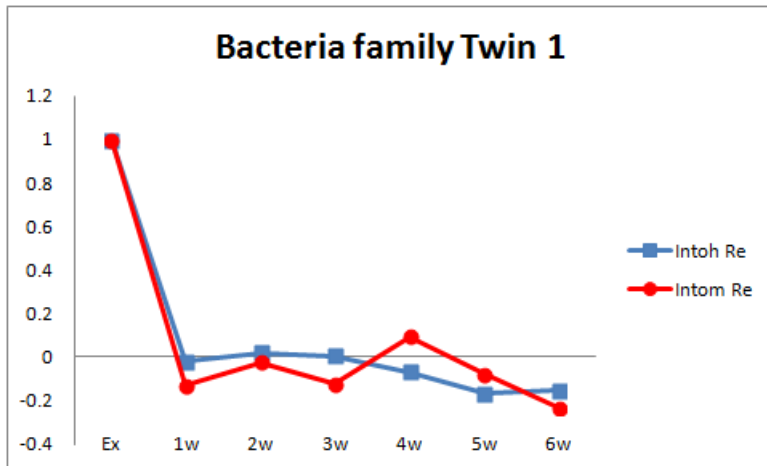
Animal-Animal

Random

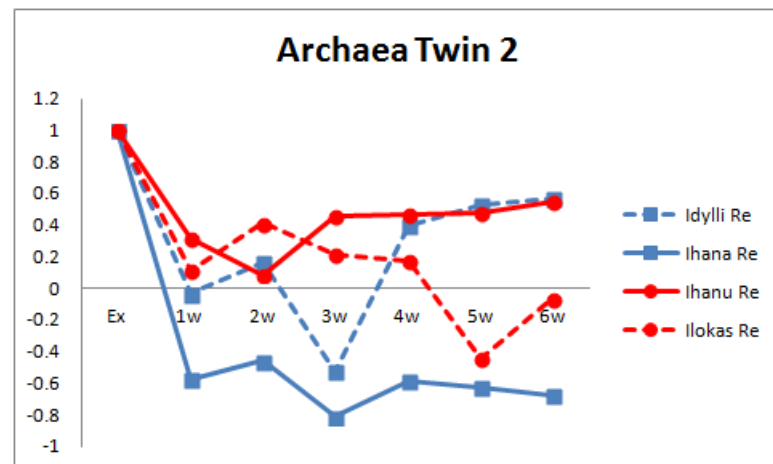
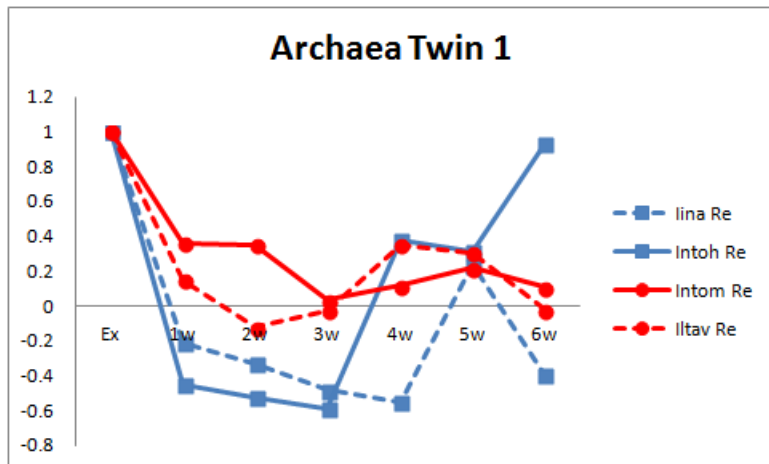
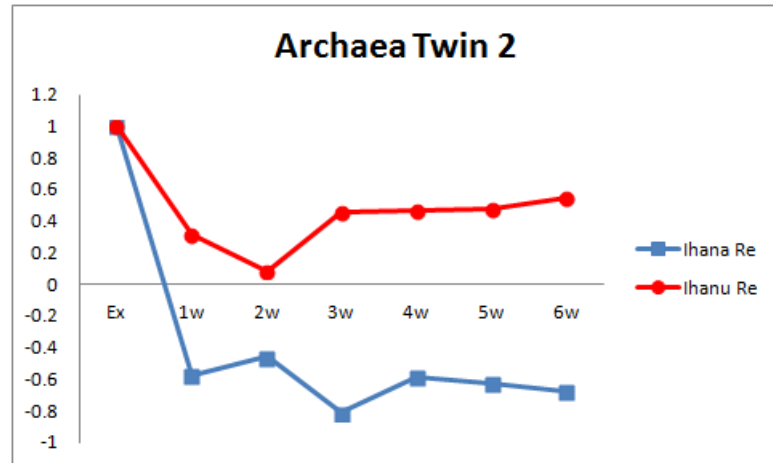
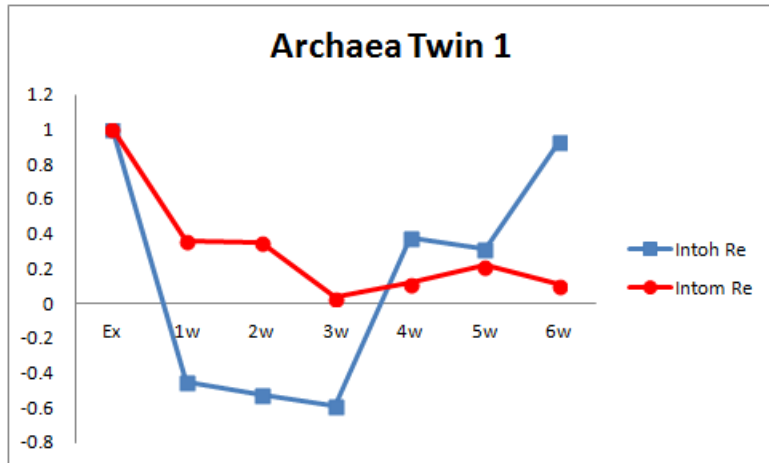
Rumen-Rumen

Calculating intraclass correlations for time points within individual

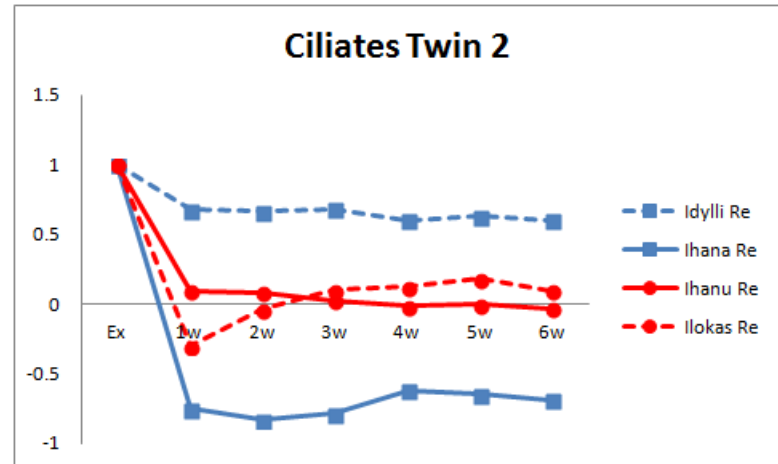
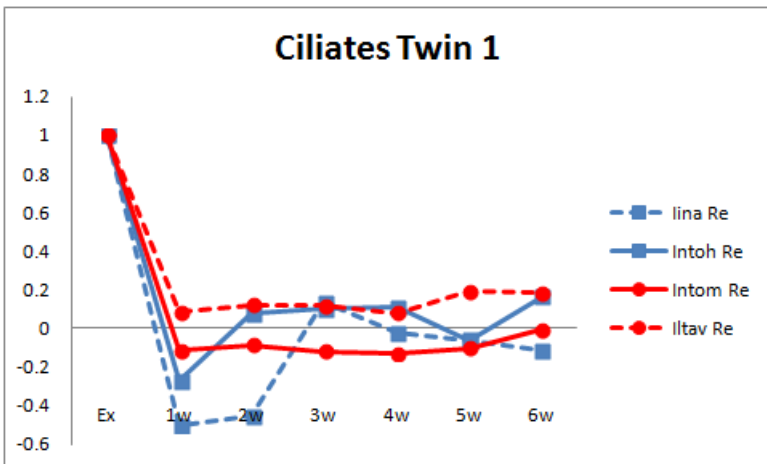
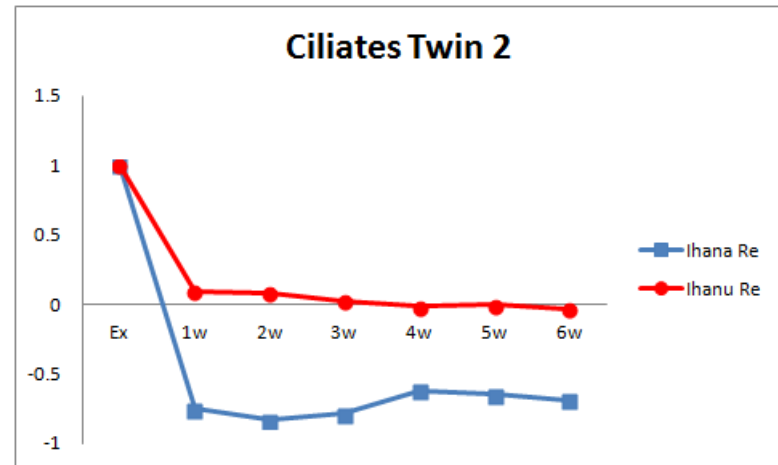
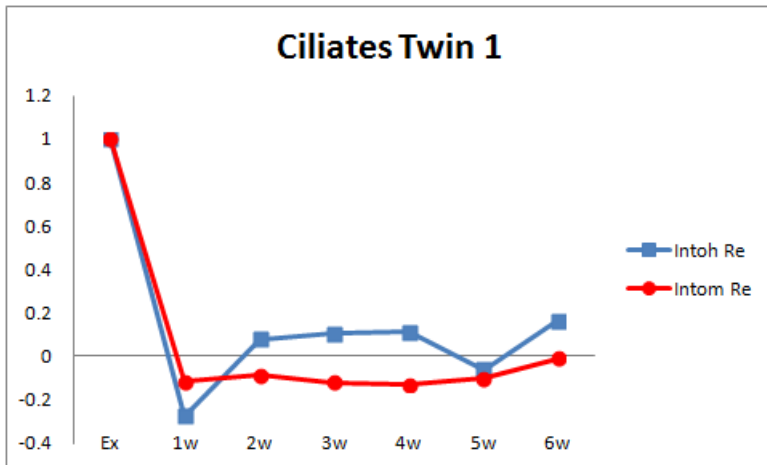
Bacteria



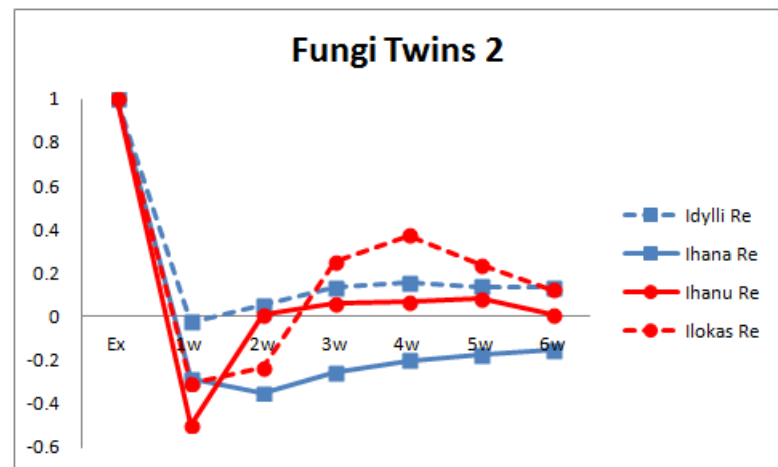
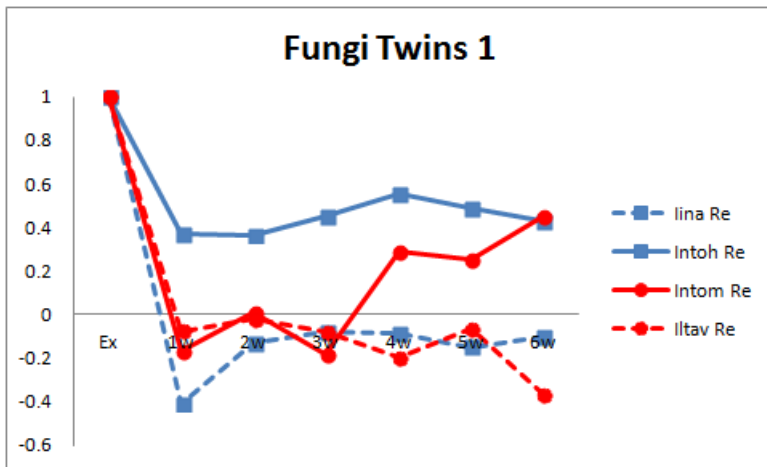
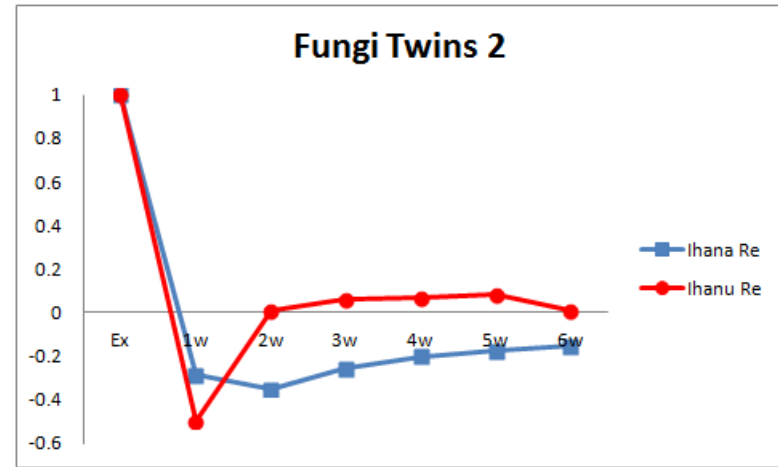
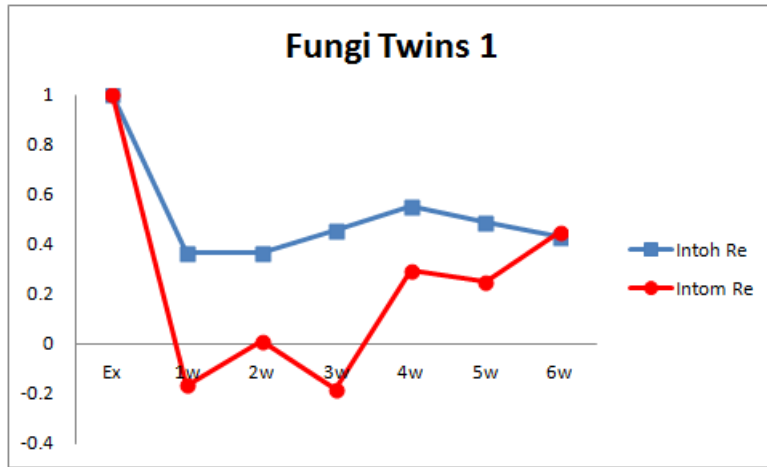
Archaea



Ciliate protozoa



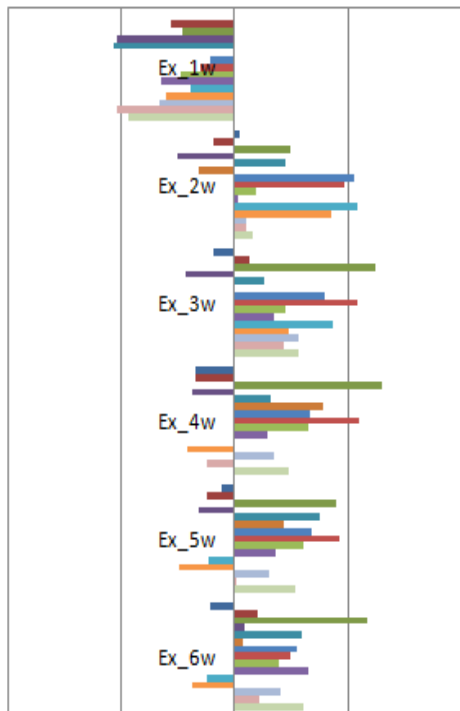
Anaerobic fungi



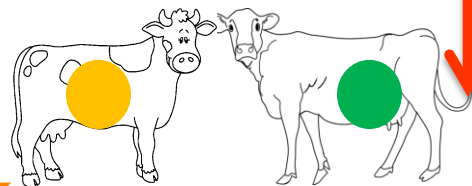
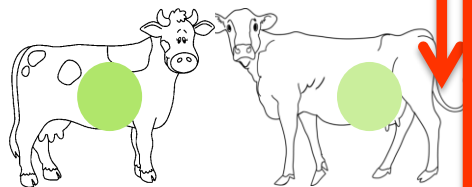
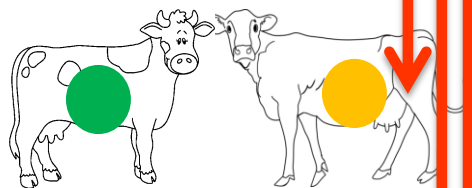
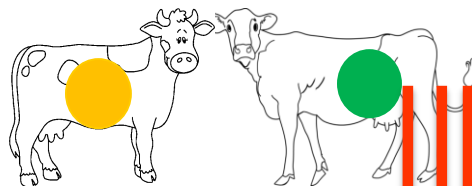
Intraclass correlation coefficient (ICC) describes how strongly individuals within a group resemble each other in terms of a particular trait (rumen microbiota)

AA<RR Fungi AA>RR

-2 -1 0 1 2

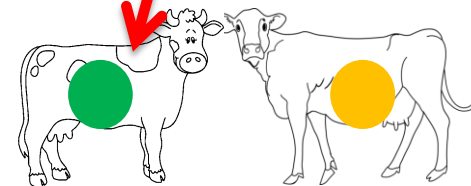
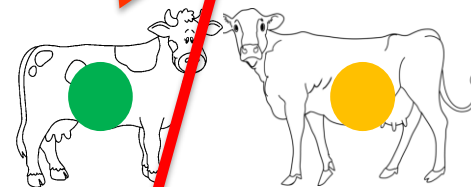
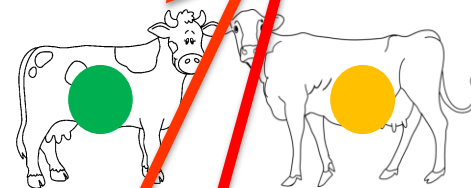
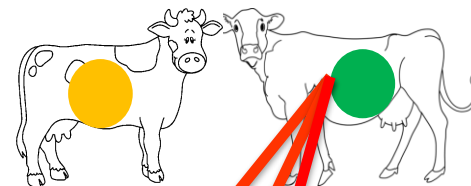


Animal-effect (AA)



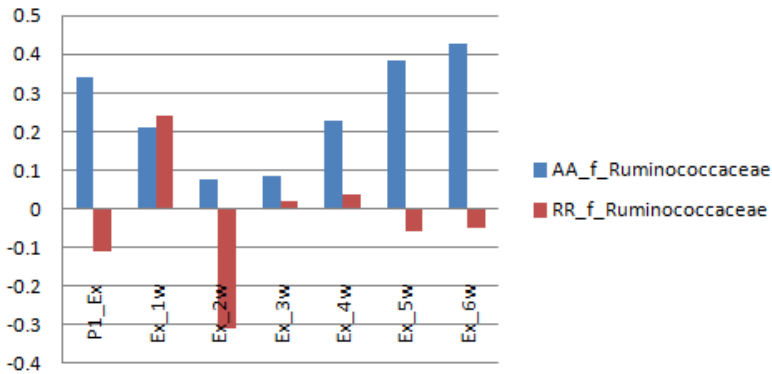
After rumen exchange

Rumen-effect (RR)

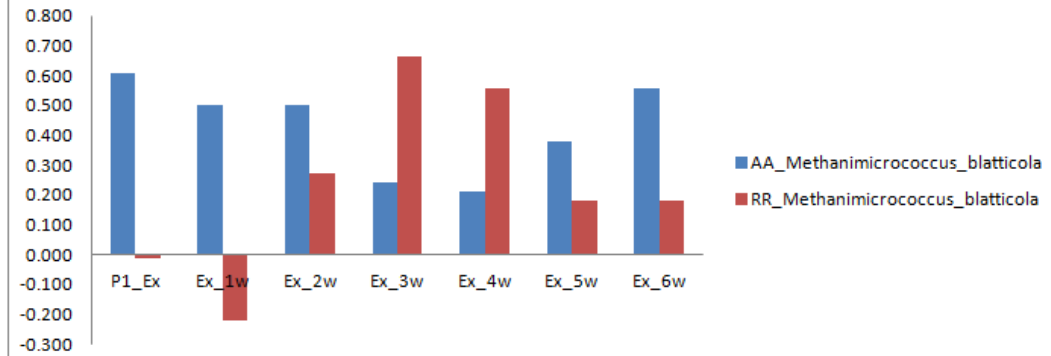


ICC correlations bacteria and archaea

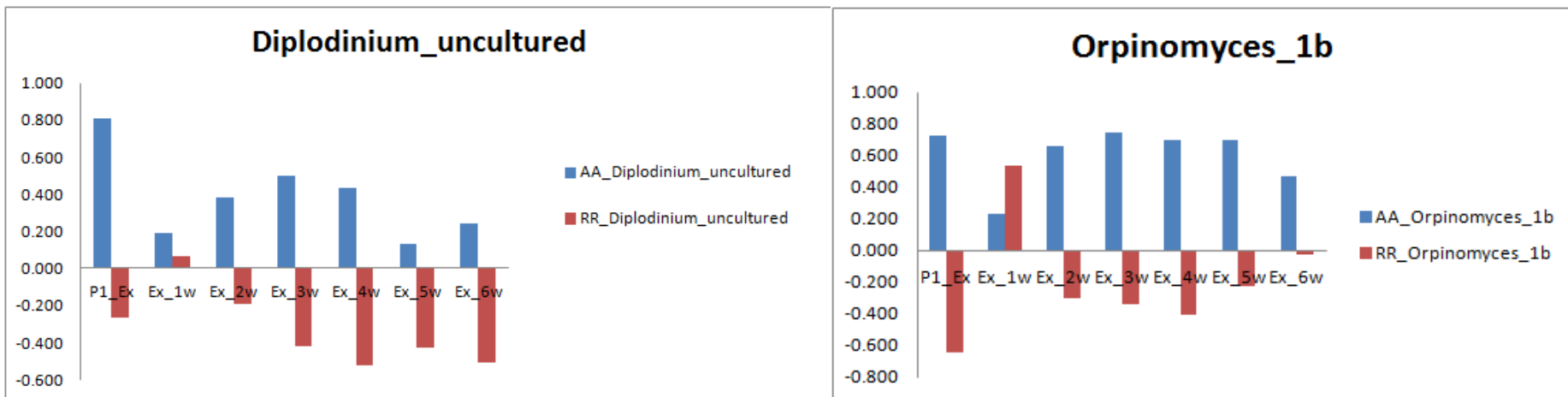
p_Firmicutes_c_Clostridia_o_Clostridiales_
f_Ruminococcaceae



Methanimicrococcus_blatticola



ICC correlations ciliate protozoa and anaerobic fungi



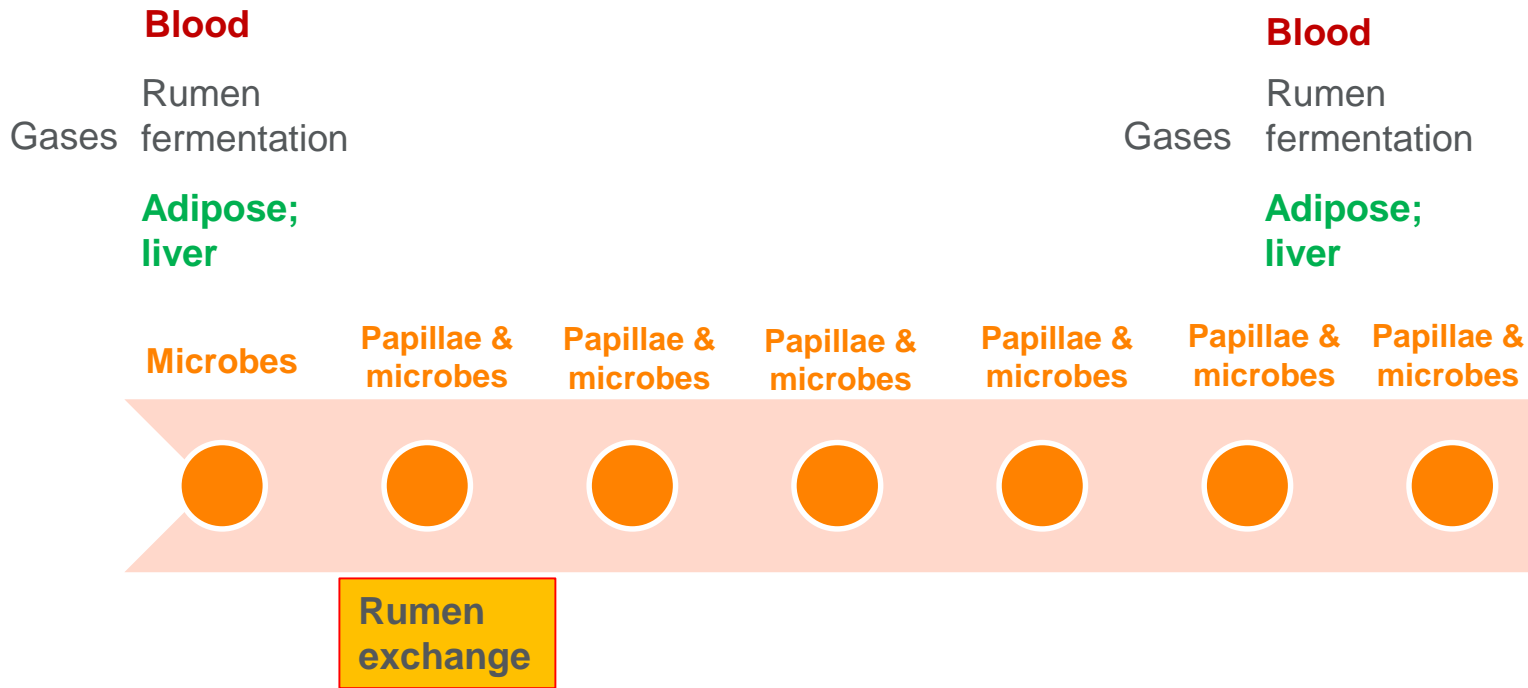
Twins - conclusions

- Rumen microbial composition in identical twins was not identical
- High between animal variation in response to rumen content exchange
- Some microbes show interesting pattern of host genetic control (e.g. Firmicutes)

Twin cow experiment - transcriptome studies

- Changes in rumen content may affect rumen epithelium function and thereby nutrient absorption kinetics
- These changes may in turn be reflected in the associated gene networks in the liver and adipose tissue
- The main objective to study the changes in the rumen epithelium, liver and adipose transcriptomes due to total exchange of rumen content/ change in microbial population

Sampling



RNA sequencing (transcriptome)

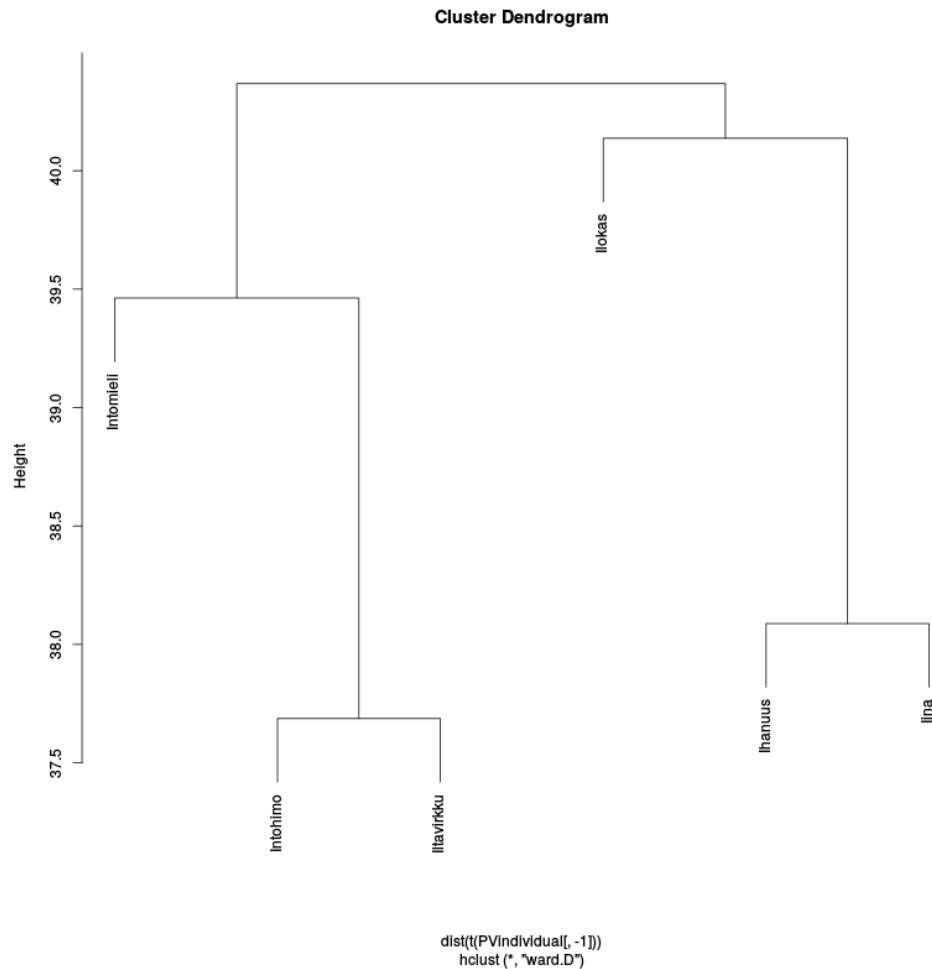
- Adipose & liver: 150 bp paired-end mRNA sequencing Illumina TruSeq/HiSeq3000;
- Average 50M reads/sample
- Papillae: 150 bp paired-end mRNA sequencing and 50 bp single-end miRNA sequencing, Illumina TruSeq
- Mapped with STAR against UMD3.1



Papillae transcriptome

- 80% of mapped reads come from top 10% genes
- Kegg pathways for these genes: Oxidative phosphorylation, nitrogen metabolism, PPAR signaling pathway, Ubiquitin mediated proteolysis, Protein processing in endoplasmic reticulum, Cholesterol biosynthesis
- high number of expressed regions, where no bovine transcripts have been annotated (50% of reads)
- checking sequences against other genomes in order to identify possible orthologs
- characterizing these regions is highly interesting for understanding the responses in the papillae (and to the annotation of the bovine genome)

Clustering of animals according to papillae gene expression changes after rumen exchange



- 27 of 108 phenotypic characters differ (Anova) between the two clusters

In Collaboration:

Luke: **Ilma Tapio**, Daniel Fischer, Seppo Ahvenjärvi, Alireza Bayat,
Heidi Leskinen, Kevin Shingfield

IAPG: Katerina Fliegerova

CNRS: Aurelie Bonin

PTP: Francesco Strozzi

Aberystwyth University: Chris Creevey

Thank you!

