



# INSIGHTS INTO THE MICROBIOTA COMPOSITION AND METATRANSCRIPTOME AT THE GUT-BODY INTERFACE

Martin Wagner<sup>1,2</sup>, Stefanie U. Wetzels<sup>1,2,3</sup>, Qendrim Zebeli<sup>2,3</sup>, Barbara Metzler-Zebeli<sup>2,3</sup>, Stephan Schmitz-Esser<sup>2,4</sup>, Evelyne Mann<sup>1,2</sup>

<sup>1</sup>Institute for Milk Hygiene, Milk Technology and Food Science,

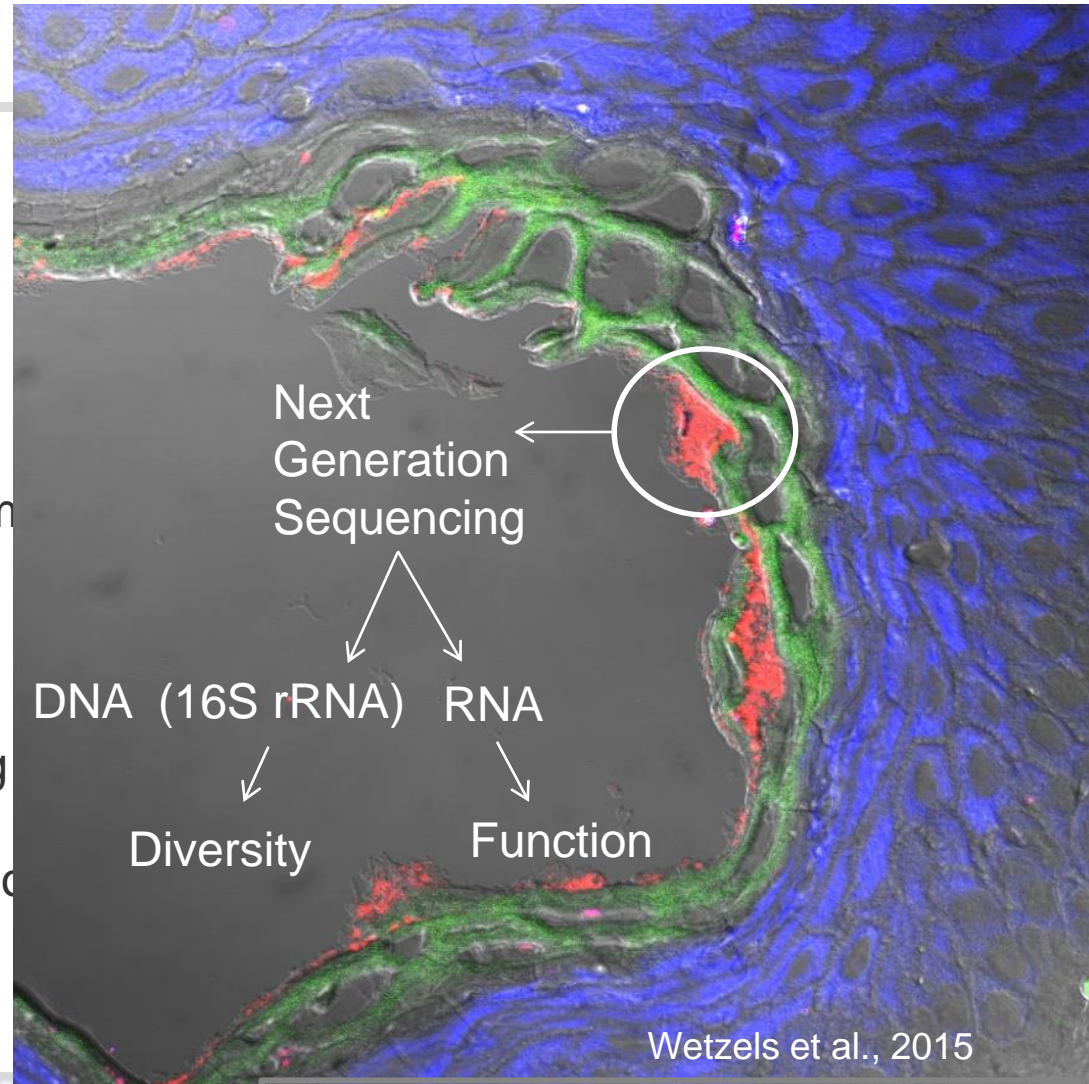
<sup>2</sup>Research Cluster 'Animal Gut Health',

<sup>3</sup>Institute of Animal Nutrition and Functional Plant Compounds, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine, Vienna, Austria,

<sup>4</sup>Department of Animal Science, Iowa State University, Ames, IA, USA.

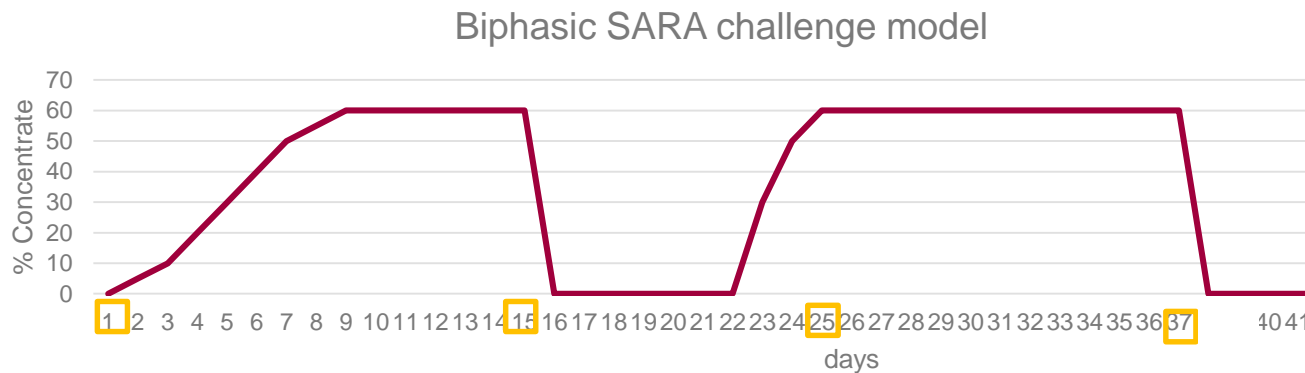
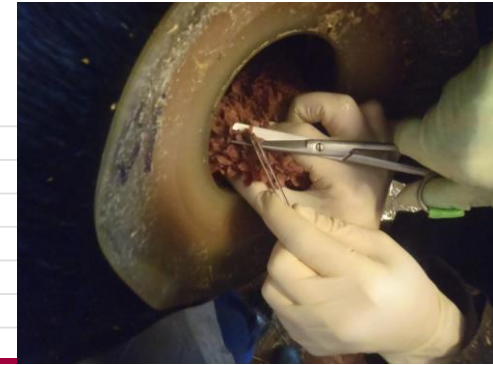
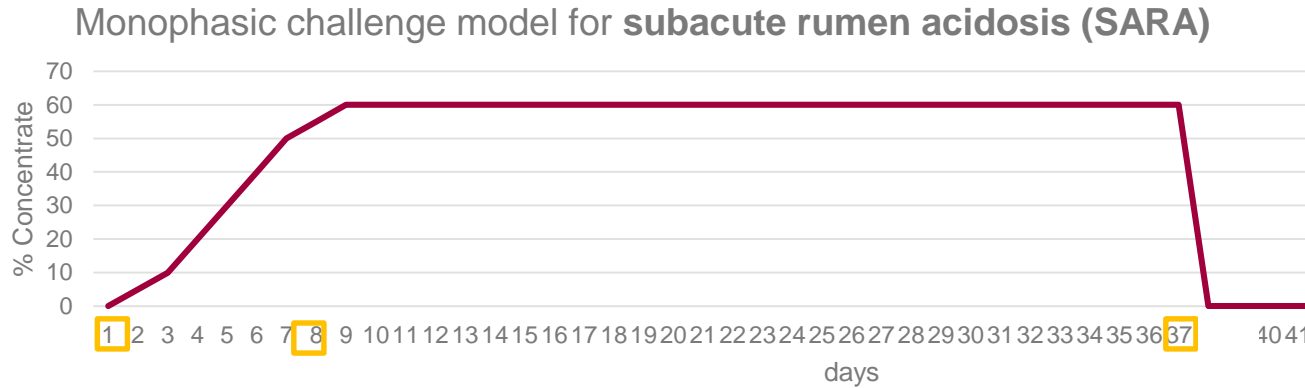
# Microbiota of the rumen wall

- $10^{13}$  bacteria/g
- 1% of ruminal bacteria attached to rumen wall (Mueller 1984)
- Multilayered keratinized epithelium
- Form protective biofilm (McCowan 1978)
- Possibly functions: hydrolysis of urea and scavenging of oxygen (Wallace 1979), tissue recycling (McCowan et al., 1978), amino acid metabolism (Mao et al., 2015)



Fluorescence-*In-Situ*-Hybridization:  
 Cy5 (blue) & green = rumen epithelium  
 Cy3 (red) = bacteria

# Study 1: How is the epimural ruminal microbiome constructed and does it contribute to metabolism?



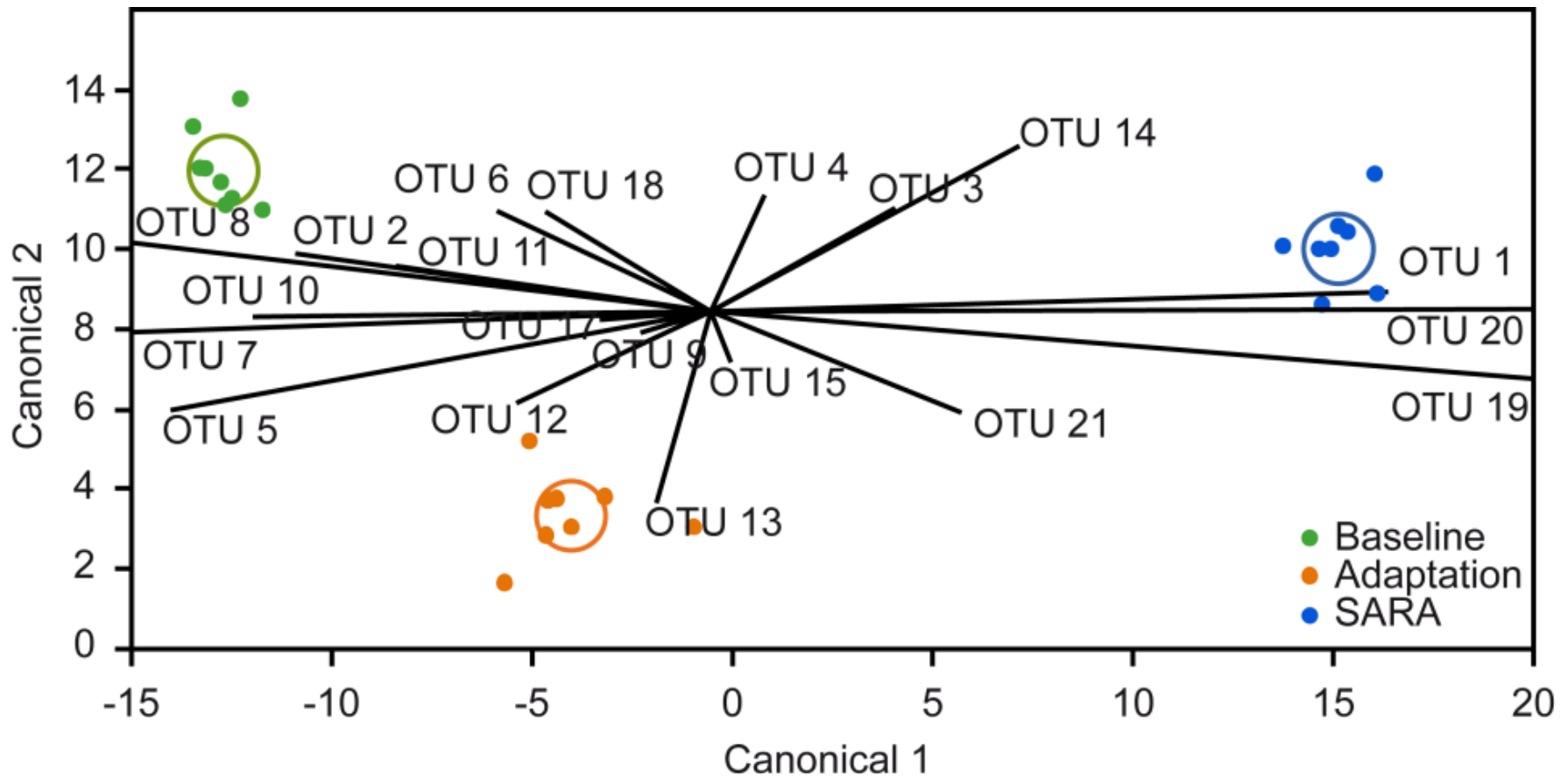
# Cows responded differently to the SARA challenge (n=8; 4 RES and 4 NRES)

| Item <sup>2</sup>            | RES    | NRES  | SEM  | P-value |
|------------------------------|--------|-------|------|---------|
| <b>Baseline (B)</b>          |        |       |      |         |
| Daily mean pH                | 6.40   | 6.44  | 0.01 | 0.82    |
| pH below 5.8 (min/d)         | 0      | 0     | 0    | NA      |
| Minimum pH                   | 6.13   | 6.19  | 0.03 | 0.27    |
| Maximum pH                   | 6.66   | 6.65  | 0.03 | 0.84    |
| Concentrate intake (kg DM/d) | 0      | 0     | 0    | -       |
| Forage intake (kg DM/d)      | 8.63   | 9.05  | 0.46 | 0.68    |
| <b>Adaptation (A)</b>        |        |       |      |         |
| Daily mean pH                | 5.92   | 6.22  | 0.09 | 0.11    |
| pH below 5.8 (min/d)         | ⇒ 495  | 135   | 132  | 0.21    |
| Minimum pH                   | ⇒ 5.28 | 5.74  | 0.14 | 0.09    |
| Maximum pH                   | 6.41   | 6.61  | 0.07 | 0.18    |
| Concentrate intake (kg DM/d) | 8.65   | 6.42  | 0.75 | 0.16    |
| Forage intake (kg DM/d)      | 7.05   | 6.71  | 0.80 | 0.85    |
| <b>SARA (S)</b>              |        |       |      |         |
| Daily mean pH                | 5.80   | 6.38  | 0.12 | 0.01    |
| pH below 5.8 (min/d)         | ⇒ 653  | 30    | 139  | 0.03    |
| Minimum pH                   | ⇒ 5.19 | 5.70  | 0.11 | 0.01    |
| Maximum pH                   | ⇒ 6.34 | 6.89  | 0.12 | 0.02    |
| Concentrate intake (kg DM/d) | 9.10   | 10.80 | 0.74 | 0.28    |
| Forage intake (kg DM/d)      | 5.48   | 6.66  | 0.59 | 0.36    |

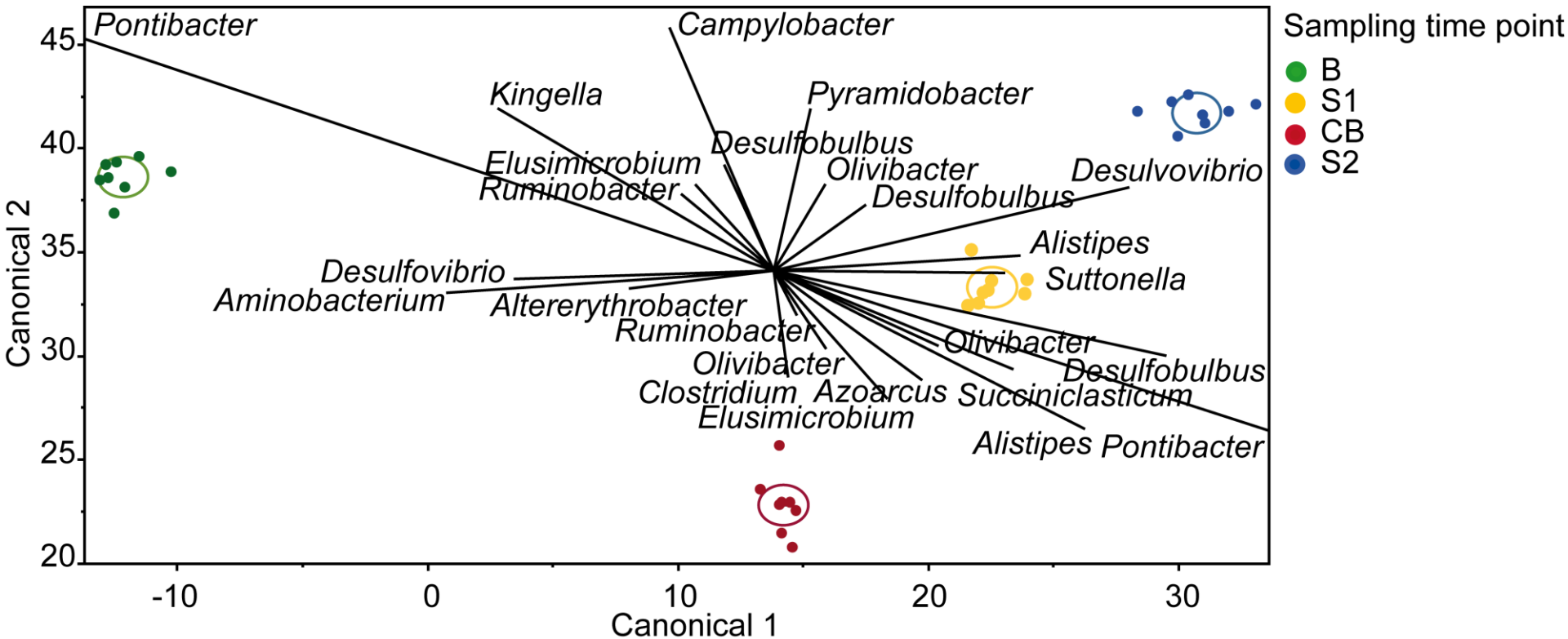
<sup>1</sup> RES were defined as cows that developed SARA (ruminal pH below 5.8 for at least 330 min/d) and NRES were defined as cows not developing SARA according to the criterion defined above.

<sup>2</sup> Baseline was 2-wk of forage feeding, Adaptation was 1-wk adaptation to SARA diet, SARA challenge was 4-wk of SARA challenge.

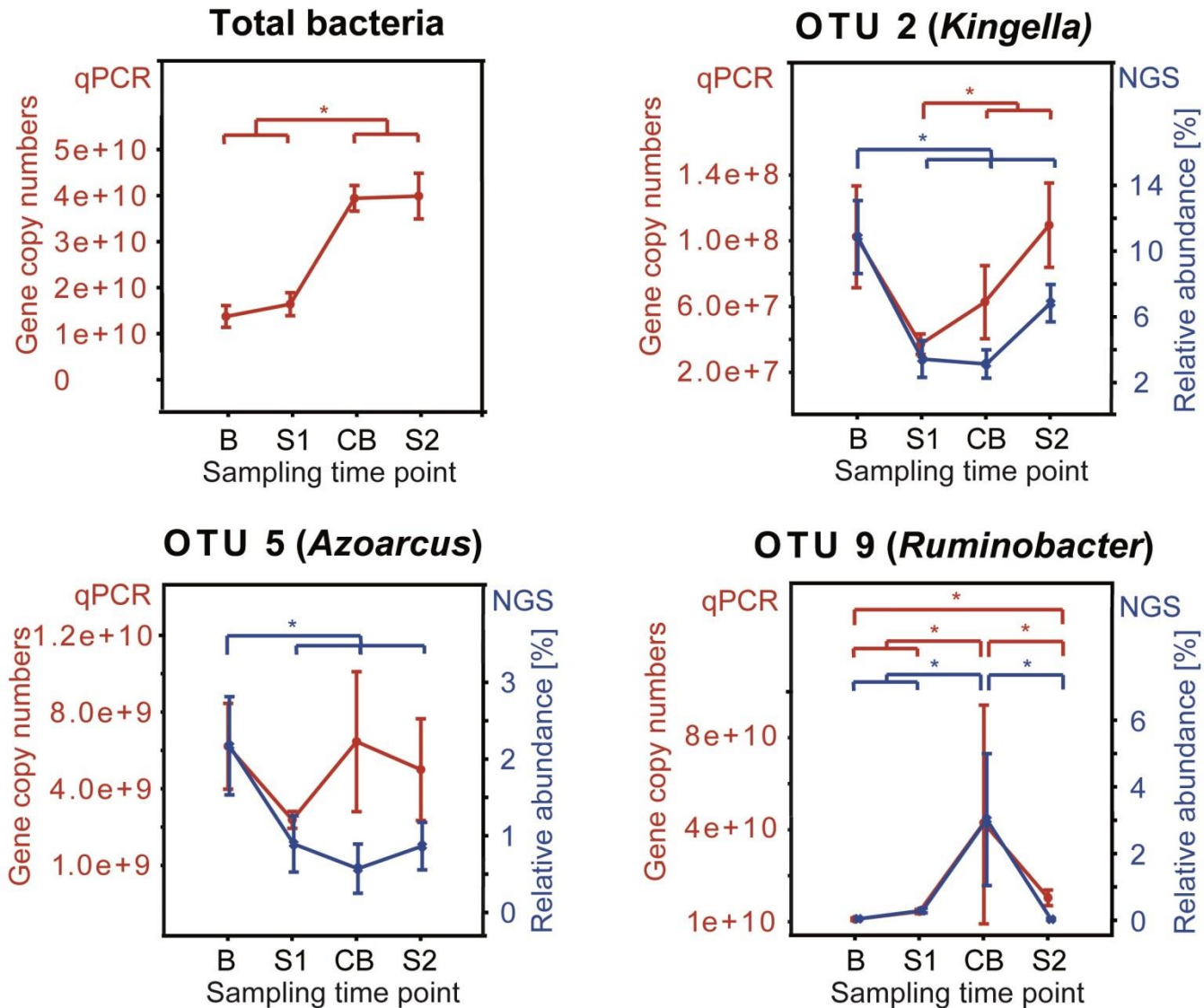
# Microbiota from each challenge period cluster separately



# A biphasic challenge drives microbiota further distinct



# Sequencing data confirmed by qPCR



# Conclusions – Diversity of the epimural microbiota

- **Microbiota of the rumen epithelium are highly diverse** from the microbiota in the lumen
- ***Campylobacter* and *Neisseriaceae*** most abundant
- **Strong shifts** in microbiota with high-concentrate feeding
  - Independently of RES/NRES affiliation
- **Different animal response** to continuous high-concentrate diet (RES/NRES)-not explainable by diversity shifts
- **Epimural microbiota after the 2<sup>nd</sup> SARA more distinct** to baseline than after 1<sup>st</sup> SARA (transient feeding model)
- **Challenge break (one week) not enough** for epimural bacterial community to recover from SARA.





# Metatranscriptome sequencing-based insights into the rumen wall microbiota gene expression (n=6; three each baseline and SARA challenge)

## Nitrogen metabolism:

- *Flavobacterium, Clostridium, Helicobacter* (Urease)
- *Campylobacter* (dissimilatory nitrate reduction)
- *Clostridium, Ruminococcus, Fibrobacter* (Nitrogenase)

## Oxydative stress response:

- *Campylobacter, Atopobium, Bifidobacterium, Clostridium, Prevotella, Fibrobacter*

## Starch metabolism and degradation of cellulose & cellobiose:

- *Verrucomicrobia, Clostridium* (was thought to be a function of luminal microbiota)

## Butyrate and propionate metabolism

- *Clostridium, Butyrivibrio, Burkholderia, Psychrobacter, Neisseria* (SCFA metabolism)



# Conclusions – Function of the epimural microbiota

**We found only few statistically significant differences between baseline and SARA in the metatranscriptome**

- Community composition shifts; compensation at functional level?
- **Functional guilds** – different strains/species within a genus may fulfil similar functions

**EM display a vital (functional) part of the metabolism of the rumen**

(Mann et al., Front. Micro. 2018)

- Housekeeping genes were among the highest expressed genes
- **Confirmed:** Nitrogen metabolism (Urease activity), Oxidative stress response
- **New:** Starch and cellulose/cellobiose degradation  
Butyrate and propionate production



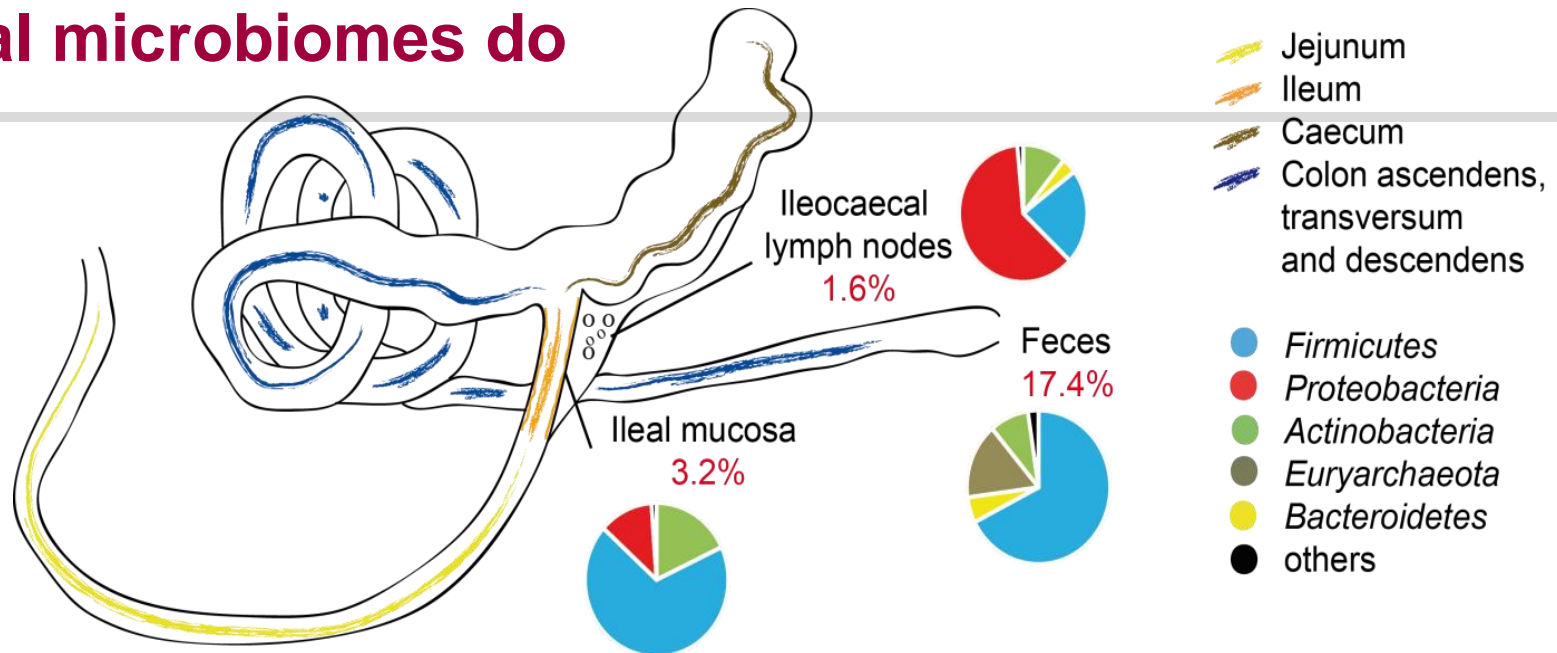
# Study 2: How does AB challenge impact on fecal microbiota, attached microbiota and lymphnodes?

- 8 pigs per group (AB and control), 3 weeks adaptation phase
- 3 weeks diet,  $\pm$  ABs (Colistin sulfate and Lincospectin)

|         |             |                          |                    |                   |
|---------|-------------|--------------------------|--------------------|-------------------|
| AB      | ICLN        | 16S rRNA gene sequencing | Cultivation        |                   |
|         | Ileum       |                          | Membrane integrity |                   |
|         | Feces-end   |                          |                    |                   |
|         | Feces-start |                          |                    |                   |
| Control | ICLN        | 16S rRNA gene sequencing | Cultivation        | Metatranscriptome |
|         | Ileum       |                          | Membrane integrity |                   |
|         | Feces-end   |                          |                    |                   |
|         | Feces-start |                          |                    |                   |



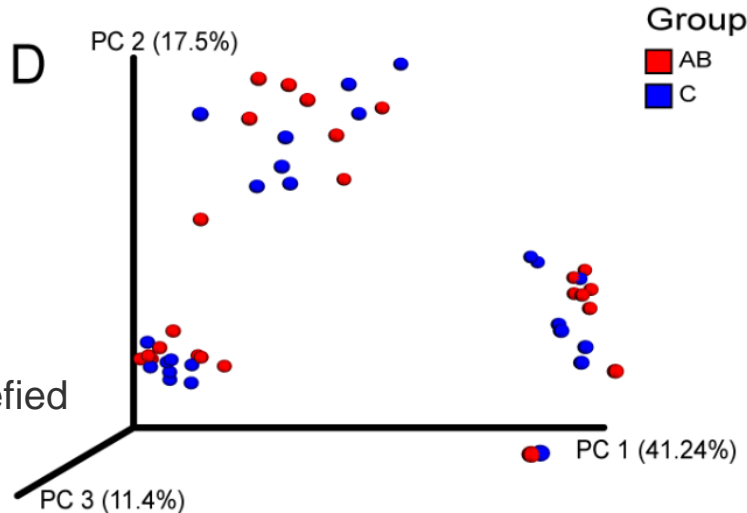
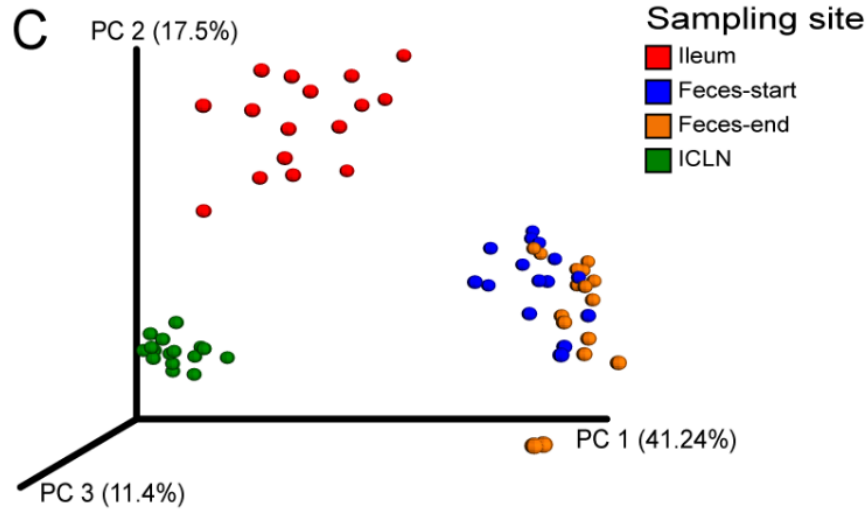
# Microbiota attached to the ileum mimick the microbiota in the lymphnodes more than fecal microbiomes do



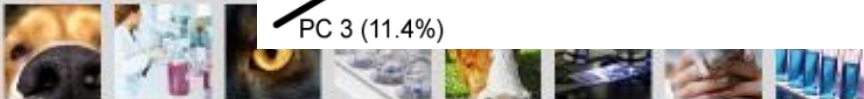
- Antibiotic treatment had a significant effect on **17.4 %** of the OTUs in feces, **3.2 %** in ileal mucosa, and **1.6 %** in ICLN samples.
- -> Bacteria might escape antibiotic treatment in lymphnodes



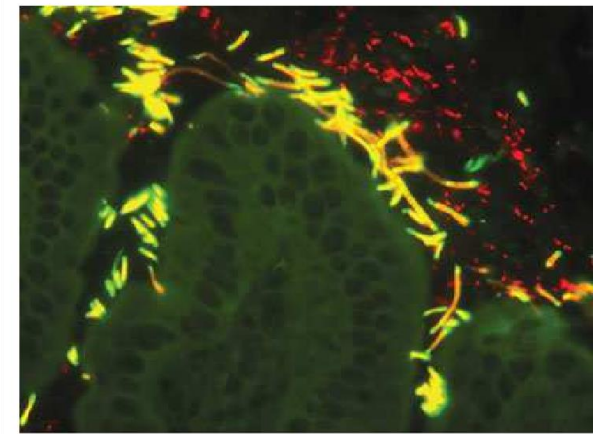
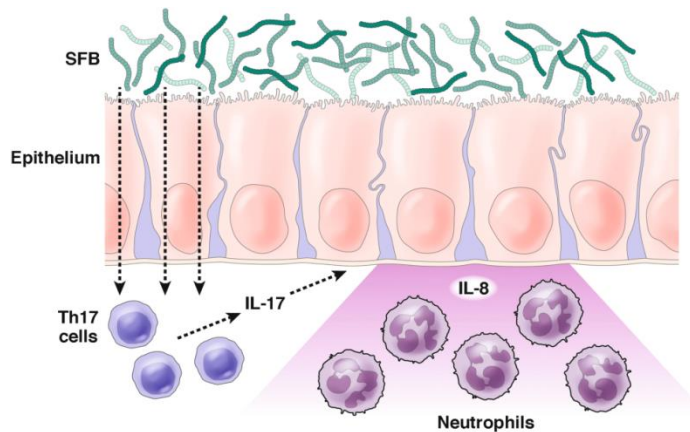
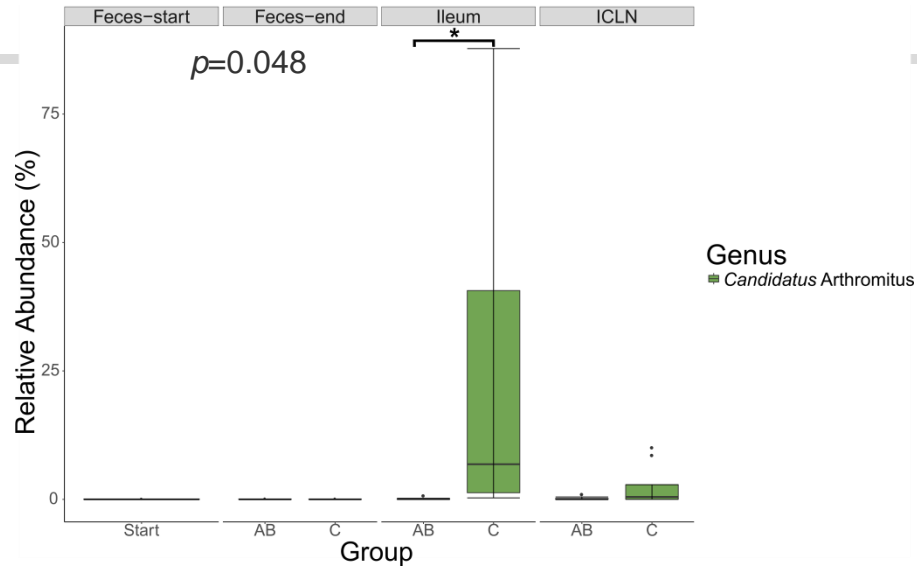
# Microbial communities separate by tissue and group



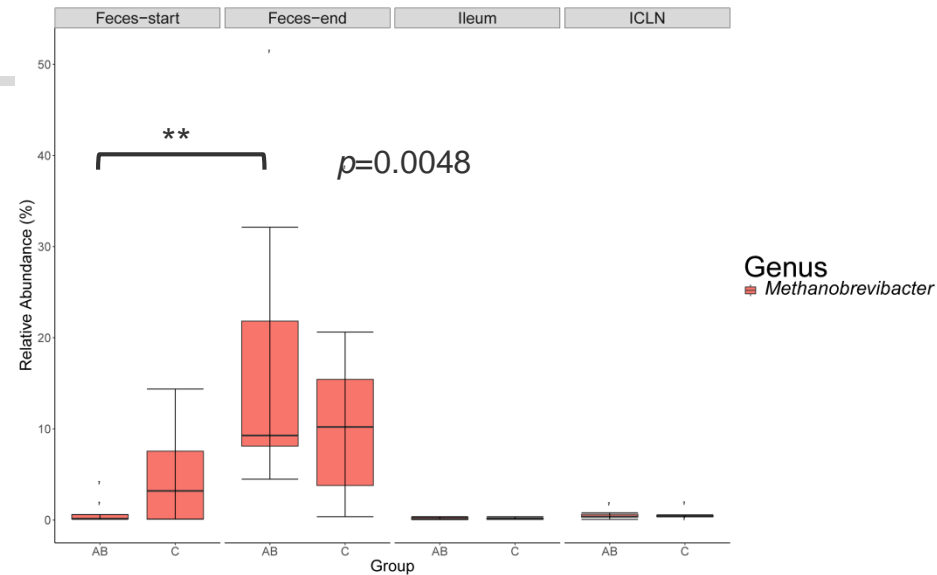
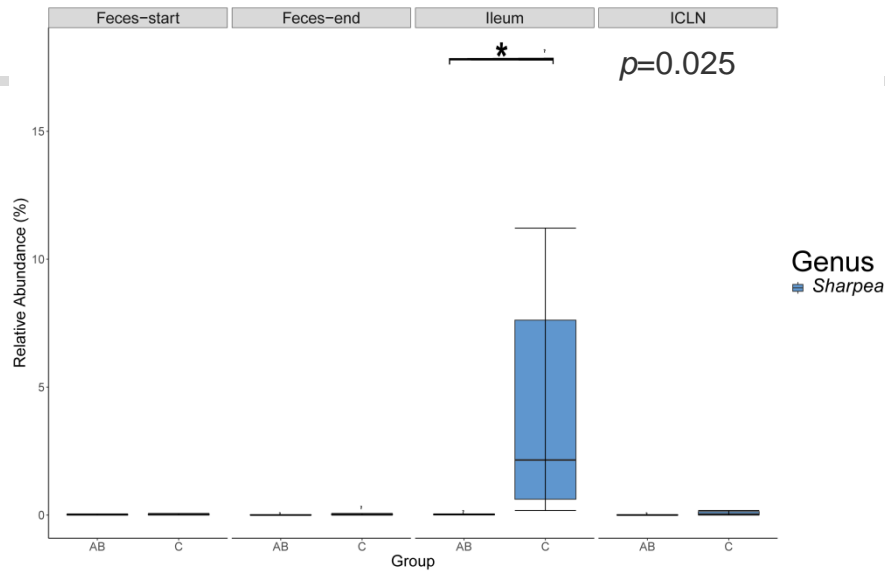
Weighted Unifrac, rarefied at 4080 sequences



# Depletion of mucosa-associated segmented filamentous bacteria



# Increased methanogenesis upon antibiotic treatment?



## Sharpea

- Gram-positive, anaerobic *Firmicutes*
- Associated to increased lactate formation (heterofermentative glycolysis) and low-methane emission (competes for H<sub>2</sub>)

## Methanobrevibacter

- Methanogenic *Archaea*
- Quickly occupying freed niches from bacteria that have been killed by the ABs
- Likely contributing to increased carbohydrate metabolism



- As for gut microbiota, microbiota of ileal mucosa and ileocaecal lymphnodes (ICLN) represent **unique corresponding environmental microbial niches**
- AB challenge had a remarkable impact on the gut (fecal) microbiome, but less impact on ileum-attached OTUs and almost none on the lymphnode microbiome-**protective microbial mechanisms involved or only a pharmacokinetic effect?**
- **Evidence that Proteobacteria (e.g. EPEC) could escape** antibiotic treatment, if they are translocated to lymph nodes (risk factor during slaughtering and meat cutting (incision))
- AB treatment of livestock might have **effects on global biogeochemical cycles**







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W|W|T|F

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