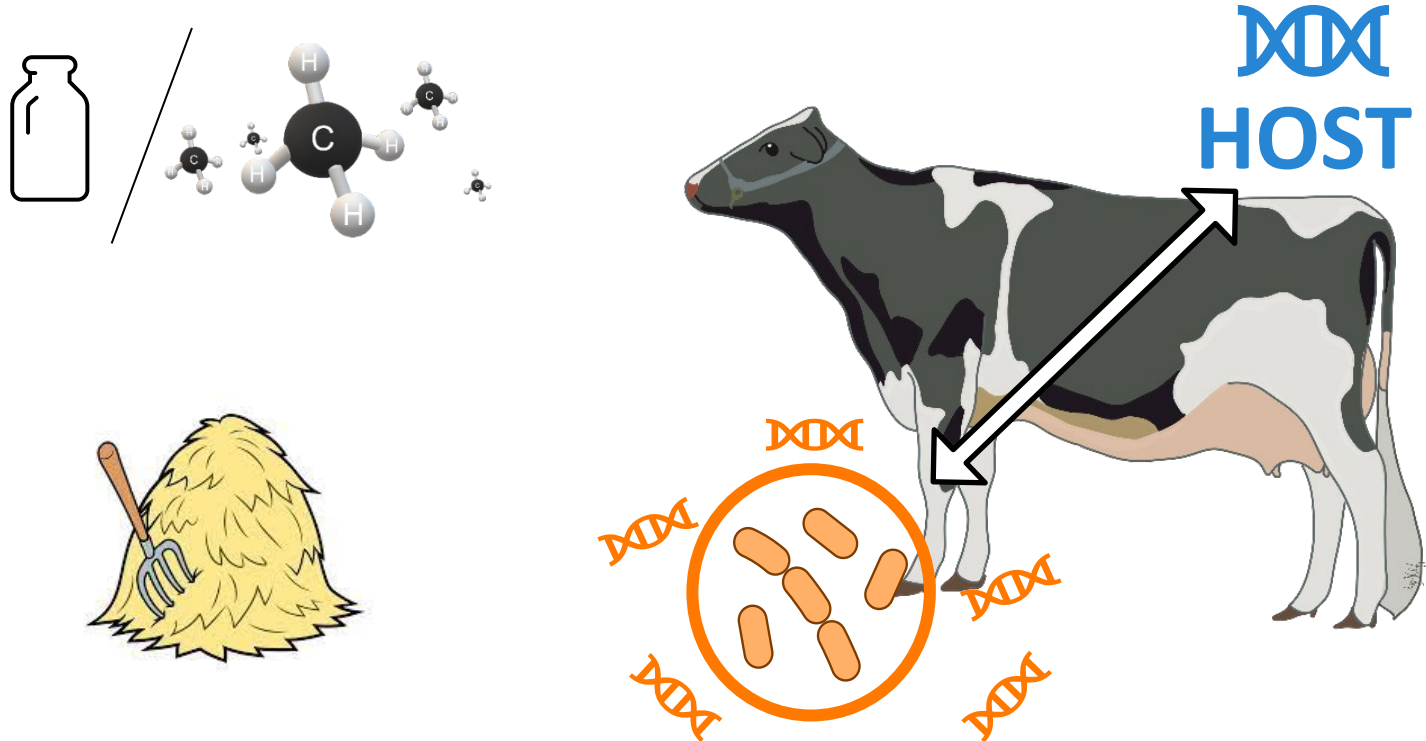


# Impact of **microbiome** on **genomic predictions** for **methane emissions** in Holstein cows

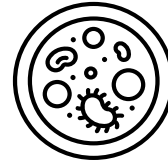
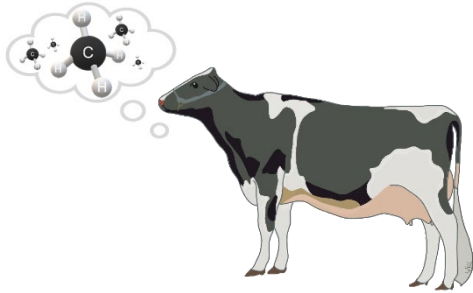
**Renzo Bonifazi**, D. Flossdorf, H. Honerlagen, B. Gredler-Grandl, M.N. Aldridge, M. Spoelstra, A.E. van Breukelen, Y. de Haas, A.C. Bouwman



# Microbiome in cattle



# Microbiome in cattle



$$\mathbf{P}_{\text{henotype}} = \mathbf{G}_{\text{enotype}} + \mathbf{M}_{\text{icrobiota}} + \mathbf{E}_{\text{nvironment}}$$

Improve prediction of (animal) performance:

- (microbial) breeding value estimation
- Improve the predictions of breeding values

# Aim

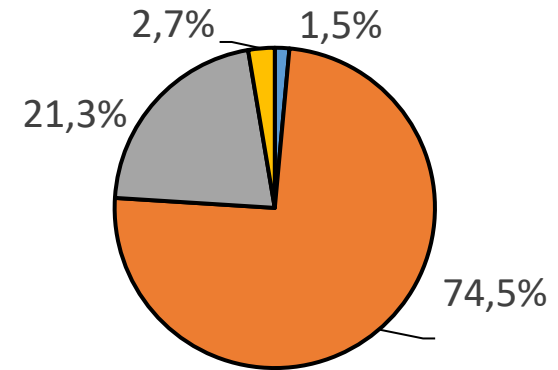
Impact of microbial composition on  
genomic predictions  
for methane emissions

# Microbiome

- 970 HOL rumen samples (12 farms in NLD)
- Long Read Sequencing → *SqueezeMeta*
- Reads count table of Taxonomical Units (OTUs):
  - Discarded: unidentified at family level
  - Discarded: *Eucaryotic* except *Fungi* and *Protozoa*
  - Included: *Viruses*
- Standardization: relative abundances (% of total OTUs reads/animal)



## Genus distribution



■ Archaea      ■ Bacteria  
■ Eukaryota    ■ Viruses

# Microbiome

Relative abundance table → Microbial Relationship Matrix (**M**)

Following *Ross et al. 2013*:

- *Log*-transformation of relative abundance profiles

$$\mathbf{X}_{[animal,OTU]} = \log(\mathbf{X}_{[animal,OTU]} + 0.01)$$

- $\mathbf{M} = \mathbf{X}\mathbf{X}'/m$  , with:

$\mathbf{X}_{[animals,OTUs]}$  = standardized *log*-transformed relative abundances

$m$  = number of OTUs (3,471)

# Methane and genotypes

- methane concentration:  $\overline{CH_4}$  in ppm per cow/day
- $CH_4$  recorded (sniffers)  $\pm 50$  days from sampling date
- Average of daily  $\overline{CH_4}$  within period ( $\geq 5$  daily  $\overline{CH_4}$  & complete fix effects)
- all animals genotyped (52K SNPs after QC)
- methane + genotypes + microbiome: 642 cows in 10 farms



|                                  | Min  | 1 <sup>st</sup> Q. | Mean  | 3 <sup>rd</sup> Q. | Max   |
|----------------------------------|------|--------------------|-------|--------------------|-------|
| cows/farm                        | 32   | 54.3               | 64.2  | 81.8               | 92    |
| $\overline{CH_4}$<br>ppm/cow/day | 87.3 | 310.5              | 436.2 | 539.5              | 863.8 |

# Scenarios implemented

| Scenario | Model                                   | BLUPs   |
|----------|---|---------|
| G        | $\overline{CH_4} \sim Xb + Zu + e$      | EBV     |
| M        | $\overline{CH_4} \sim Xb + Wm + e$      | MBV     |
| G+M      | $\overline{CH_4} \sim Xb + Zu + Wm + e$ | EBV+MBV |

**Fixed effects:** herd, lactation stage (4 classes based on DIM), parity (1 to 4+),  $\overline{CO_2}$  (as covariate)

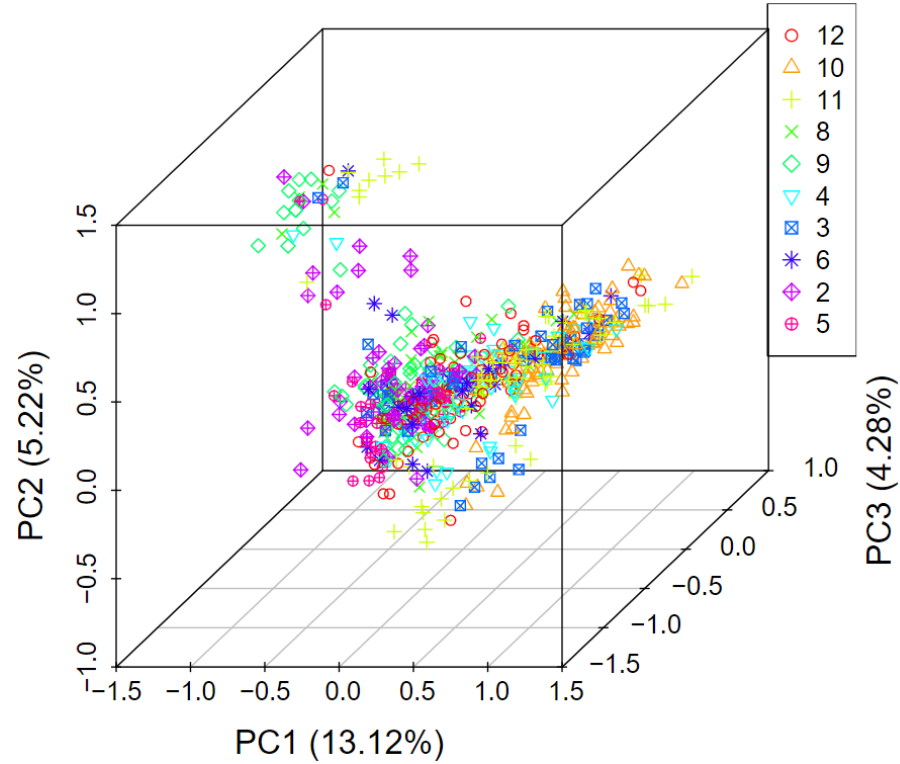
**Software:** Custom R pipeline + ASReml (VCE and BVE)

# Validation

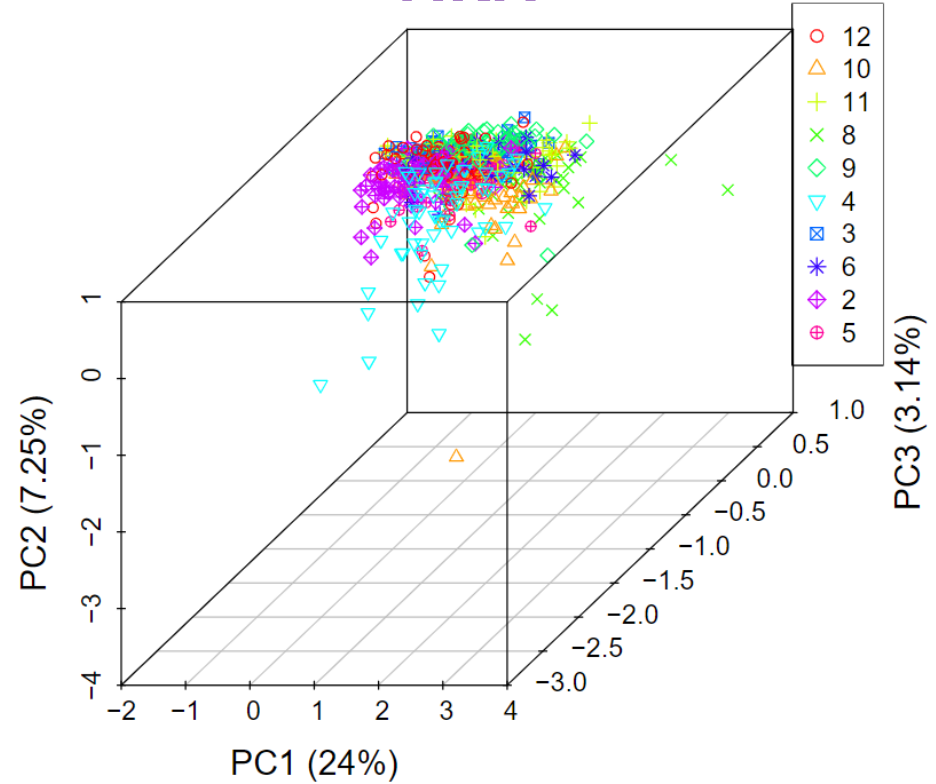
- Leave-one-farm-out (limited dataset)
- EBVs and MBVs compared with  $y^*$  from most complete model (G+M)
- $y^* = EBV + MBV + e$
- Prediction accuracy:  $cor(y^*, BV) / \sqrt{h^2 + m^2}$
- Dispersion: regression slope
- SE via bootstrapping

# PCA

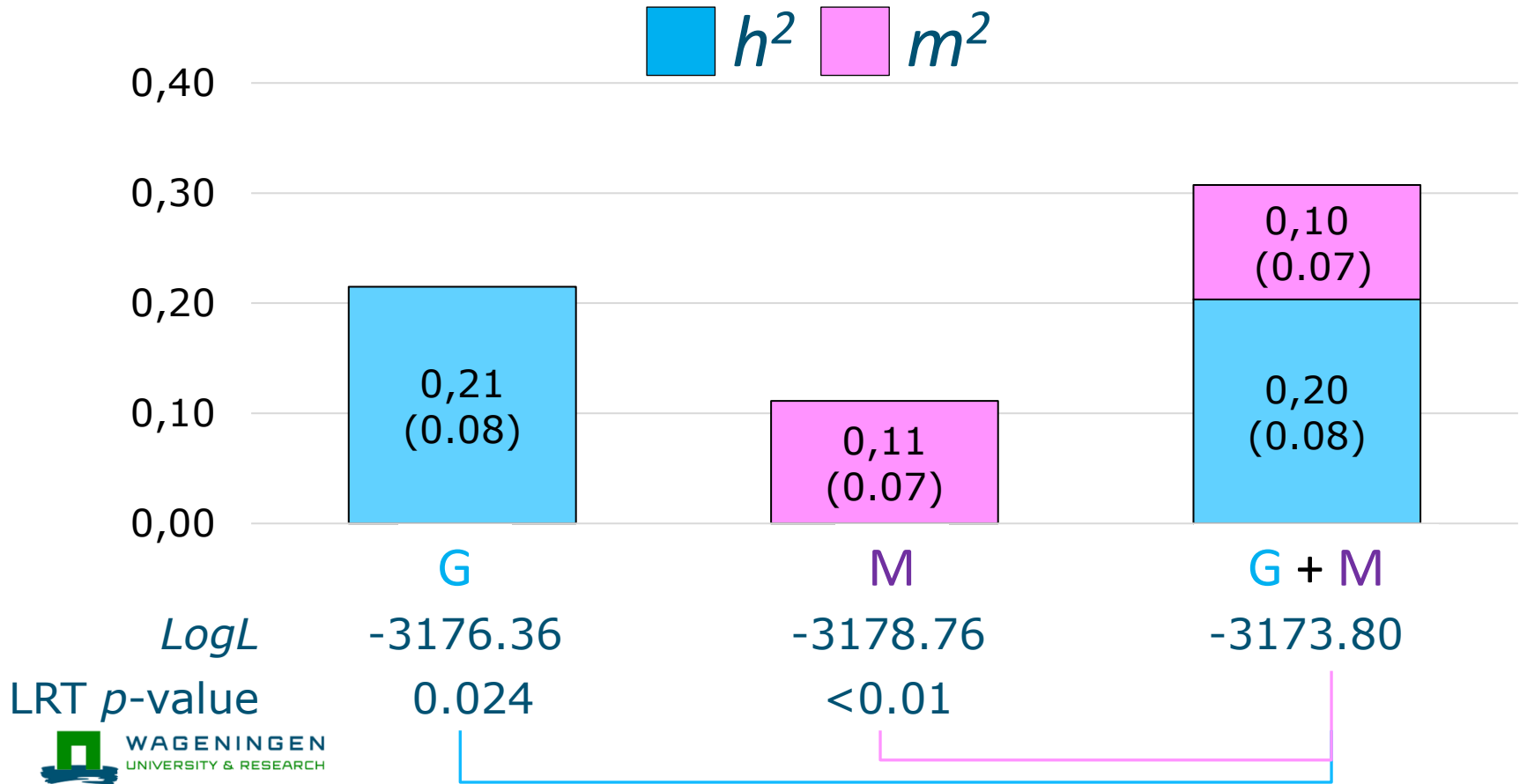
## GRM



## MRM



# Results – estimated VC



# Results – validation

(SE in brackets)

## Prediction accuracies

|     | $cor(EBV, y^*)$ | $cor(MBV, y^*)$ | $cor(EBV + MBV, y^*)$ |
|-----|-----------------|-----------------|-----------------------|
| G   | 0.07 (0.07)     | -               | -                     |
| M   | -               | 0.31 (0.06)     | -                     |
| G+M | 0.07 (0.07)     | 0.31 (0.06)     | 0.24 (0.07)           |

## Dispersion

|     |             |             |             |
|-----|-------------|-------------|-------------|
| G+M | 0.31 (0.28) | 1.70 (0.34) | 0.80 (0.23) |
|-----|-------------|-------------|-------------|

# Take-home messages

- $\overline{CH_4}$  ppm/cow/day :  $h^2 = 0.20$ ,  $m^2 = 0.10$
- Prediction accuracies:
  - Low using only **G** (0.07) (small dataset)
  - Good using only **M** (0.31)
  - Improved moving from **G** to **G+M** ( $\rightarrow 0.24$  & less dispersion)

# Acknowledgements

Mario Calus, Henk Sulkers, Larissa Zetouni



**Re-Livestock**  
RESILIENT FARMING SYSTEMS



Ministerie van Landbouw,  
Natuur en Voedselkwaliteit

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## Thanks for your attention



# Take-home messages

- $\overline{CH_4}$  ppm/cow/day :  $h^2 = 0.20$ ,  $m^2 = 0.10$
- Prediction accuracies:
  - Low using only **G** (0.07) (small dataset)
  - Good using only **M** (0.31)
  - Improved moving from **G** to **G+M** ( $\rightarrow 0.24$  & less dispersion)

# Additional slides

# Results – validation

(SE in brackets)

## Prediction accuracies

|     | $cor(EBV, y^*)$ | $cor(MBV, y^*)$ | $cor(EBV + MBV, y^*)$ |
|-----|-----------------|-----------------|-----------------------|
| G   | 0.07 (0.07)     | -               | -                     |
| M   | -               | 0.31 (0.06)     | -                     |
| G+M | 0.07 (0.07)     | 0.31 (0.06)     | 0.24 (0.07)           |

## Dispersion

|     | $cor(EBV, y^*)$ | $cor(MBV, y^*)$ | $cor(EBV + MBV, y^*)$ |
|-----|-----------------|-----------------|-----------------------|
| G   | 0.27 (0.27)     | -               | -                     |
| M   | -               | 1.70 (0.34)     | -                     |
| G+M | 0.31 (0.28)     | 1.70 (0.34)     | 0.80 (0.23)           |