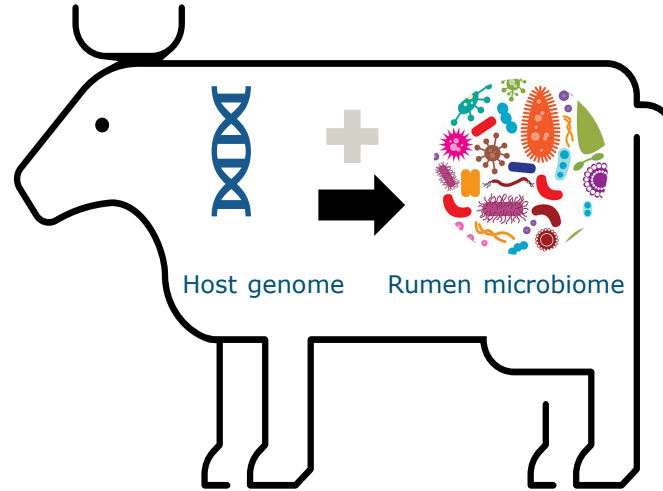
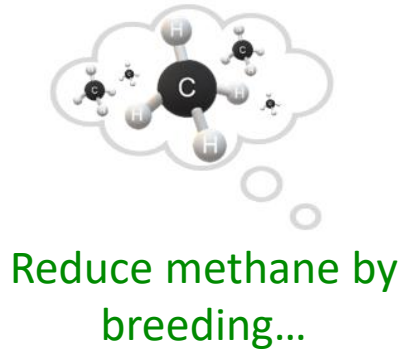


Adding **microbial data** to enhance **breeding** for **lower methane** emissions

Hanne Honerlagen, Renzo Bonifazi, David Flossdorf, Aniek Bouwman

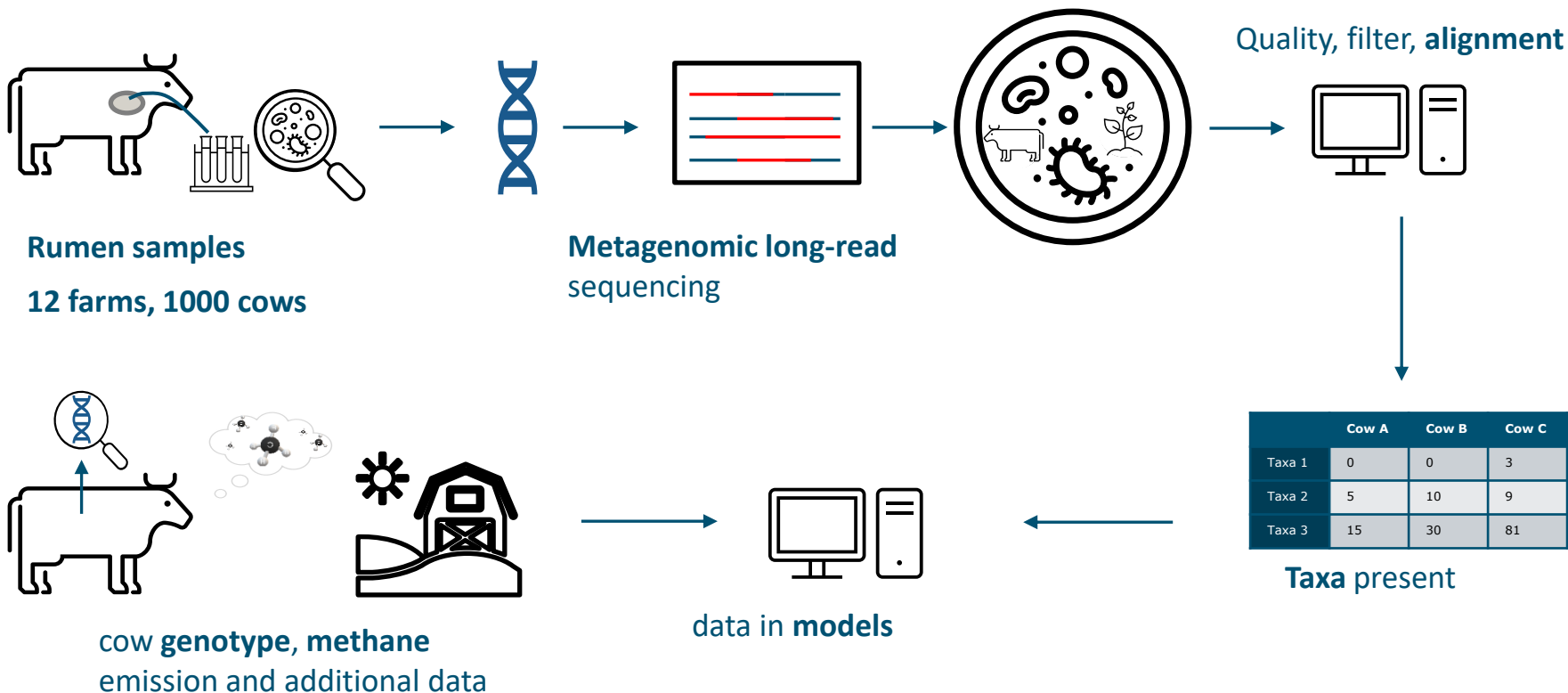


Microbial- enhanced selection to lower methane?



1. Can rumen microbial information help to predict methane phenotypes?
2. Which rumen microbial taxa are potentially targeted by breeding for lower methane?

Data collection



What has been done

1. Can rumen microbial information help to predict methane phenotypes?

❑ Prediction of methane phenotypes by 3 models using:

1. Host genetic data (G)

2. Microbial data (M)

3. Host genetic + Microbial data (G+M)

Model	Equation
G	$\overline{CH_4} \sim Xb + Zu + e$
M	$\overline{CH_4} \sim Xb + Wm + e$
G+M	$\overline{CH_4} \sim Xb + Zu + Wm + e$

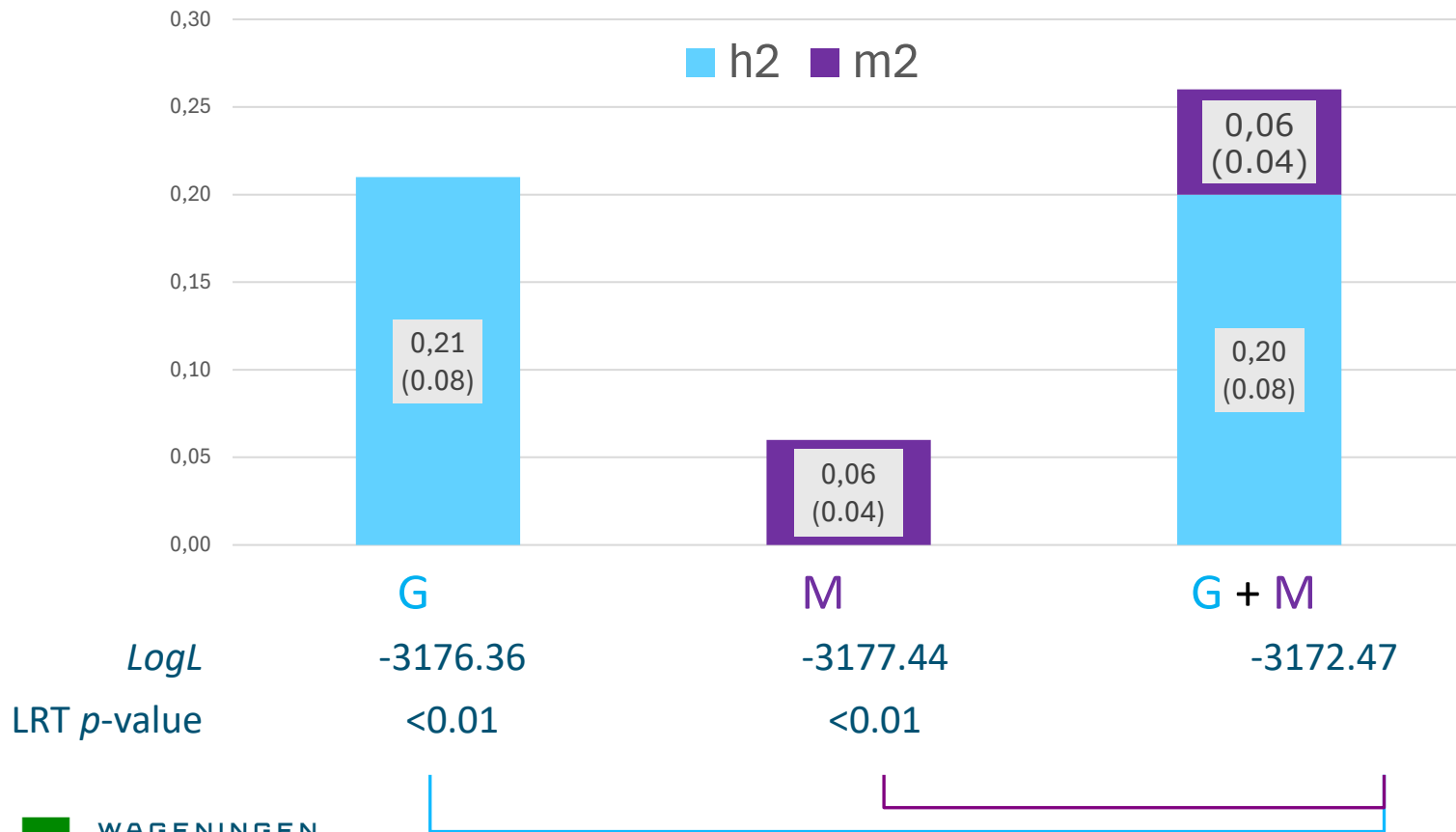
$\overline{CH_4}$ based on CH₄ ppm averages per cow/day ±50 days from rumen fluid sampling date

Xb fixed effects of herd, lact. stage (4 classes), parity (1 to 4+), CO₂ (as covariate)

Zu modeled using a genomic relationship matrix based on 52K SNPs

Wm modeled using a microbial relationship matrix based on 577 genera (Ross *et al.*, 2013)

Results – CH4 variance explained



Prediction accuracies

	$cor(EBV, Y^*)$	$cor(EMV, Y^*)$	$cor(EBV+EMV, Y^*)$
G	0.04 (0.04)	-	-
M	-	0.17 (0.03)	-
G+M	0.04 (0.04)	0.17 (0.03)	0.15 (0.04)

(SE in brackets)

Dispersion

G+M	0.29 (0.28)	1.78 (0.37)	0.77 (0.23)
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Take-home messages research question 1:

1. Can rumen microbial information help to predict methane phenotypes?

- $\overline{CH_4}$: $h^2 = 0.21$, $m^2 = 0.06 \rightarrow m^2$ low
- Prediction accuracies:
 - Low using only **G** (~ 0.04) (small dataset)
 - Good using only **M** (~ 0.17)
 - Improve moving from **G** to **G+M** ($\rightarrow 0.15$ & less dispersion)

What has been done: Roadmap

2. Which rumen microbial taxa are potentially targeted by breeding for lower methane?

❑ Heritabilities of rumen microbial genera

$$\text{microbe} = Xb + Zu + e$$

microbe = rel. abundance of genus in the animal

Xb herd, lactst, parity, CO₂

Zu based on 52K SNPs

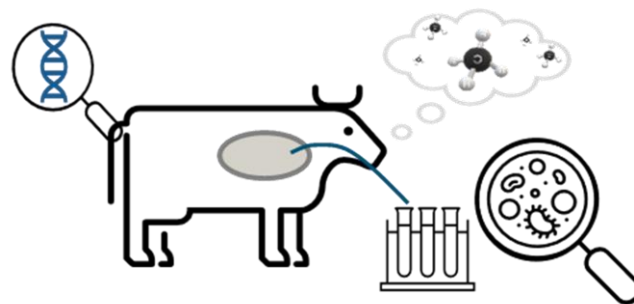
❑ Associations of microbial genera to methane

$$\overline{CH_4} = Xb + Zu + e$$

$\overline{CH_4}$ = CH₄ ppm averages per cow/day ±50 days from sampling date

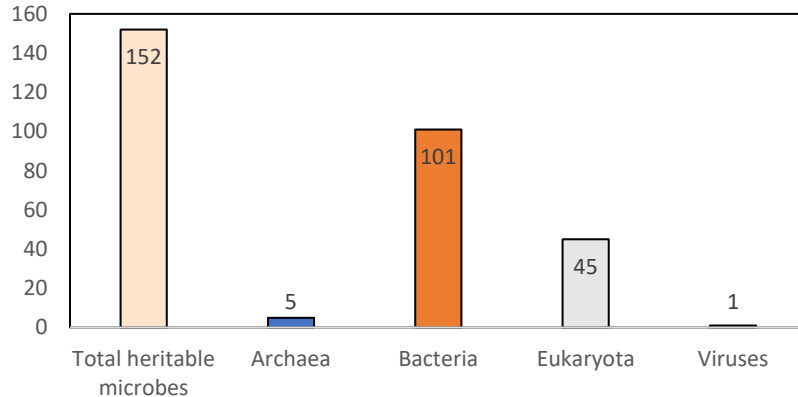
Xb herd, lactst, parity, CO₂, **microbe** (as covariate)

Zu based on 52K SNPs



Heritability and association with methane

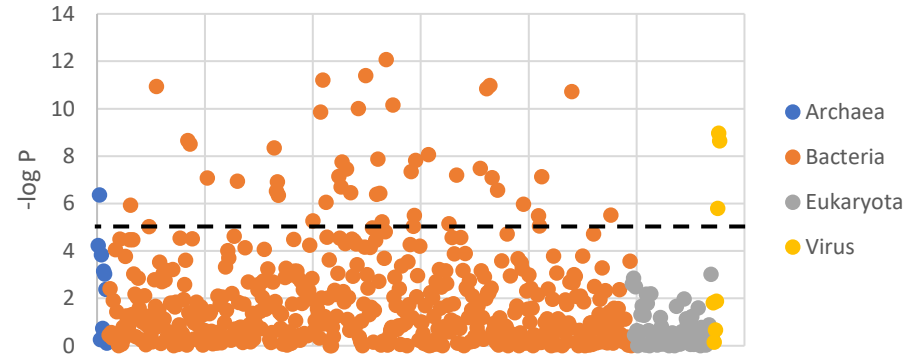
Heritable genera found in core microbiome



148 / 577 genera heritable*

* h^2 = significantly different from zero

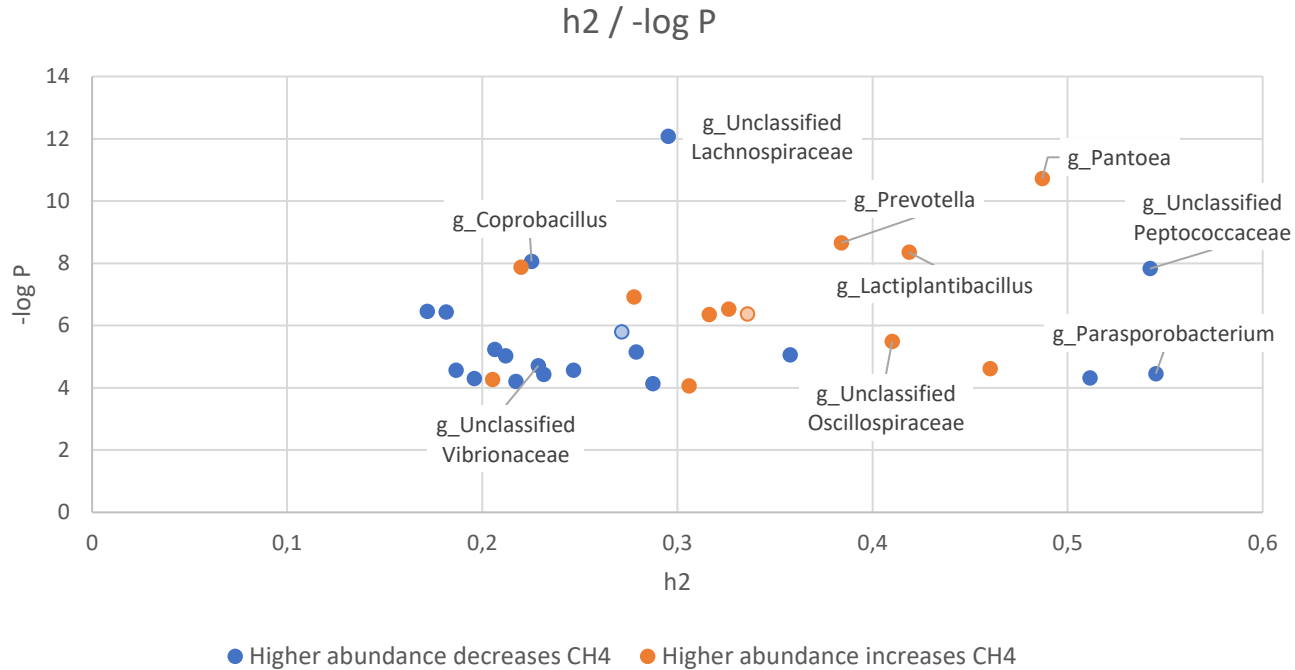
Association of genera with methane



80 / 577 genera associated* with methane

*significance threshold $-\log_{10}(p) > 4$

Heritable genera & associated with methane



Take-home messages research question 2:

2. Which rumen microbial taxa are potentially targeted by breeding for lower methane?

- Heritabilities: 148 genera heritable, h^2 up to 0.57
- Association: 80 genera associated with methane

➤ In total, 30 microbial genera potentially targeted by breeding for lower methane.

Thank you for your attention!

This work is part of the Re-Livestock EU Horizon project and the Knowledge and Innovation Agenda of the Dutch Ministry of Agriculture, Nature, and Food Quality



Ministerie van Landbouw,
Natuur en Voedselkwaliteit



Re-Livestock

RESILIENT FARMING SYSTEMS



WAGENINGEN
UNIVERSITY & RESEARCH



Contact: Hanne Honerlagen

hanne.honerlagen@wur.nl

Tel/SMS/Whatsapp: +31 6 81172123



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: $cor(Y^*, x) / \sqrt{h^2 + m^2}$
- Dispersion: regression slope
- SE via bootstrapping

Next steps / current work

- Calculate genetic covariances between these 30 genera and CH4
- Use parameters in selection index to estimate their potential additional genetic gain

Microbial relationship matrix

Relative abundance table → Microbial Relationship Matrix (**M**)
Following *Ross et al. 2013*:

- *Log*-transformation on relative abundance profiles

$$\mathbf{X}_{\text{anim, taxon}} = \log(\mathbf{X}_{\text{anim, taxon}} + 0.01)$$

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

with:

$\mathbf{X}_{\text{anim, taxon}}$ = standardized count matrix

m = number of microbial taxa (577 genera)

Data: Methane and genotypes

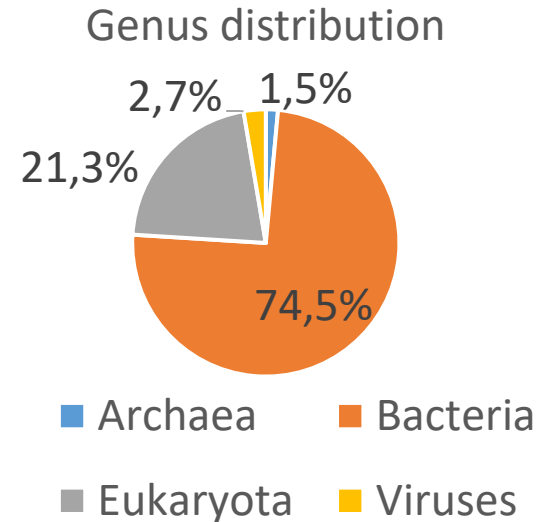
- methane concentration: $\overline{CH_4}$ in *ppm per cow/day*
- recorded (sniffers) ± 50 days from sampling date
- at least 5 visits/week/cow and complete fixed effects (also $\overline{CO_2}$)
- all animals were genotyped at 52K SNPs (after QC)
- methane + genotypes + microbiome: 642 cows in 10 farms



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

Data: Microbiome

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

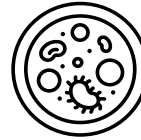
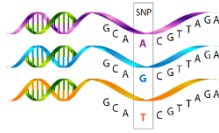
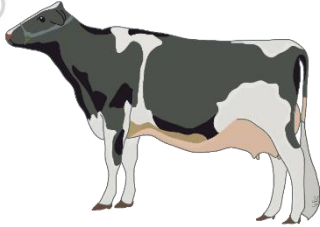


More details: *Flossdorf D.* @10.00

Thank you.



Microbiome in cattle

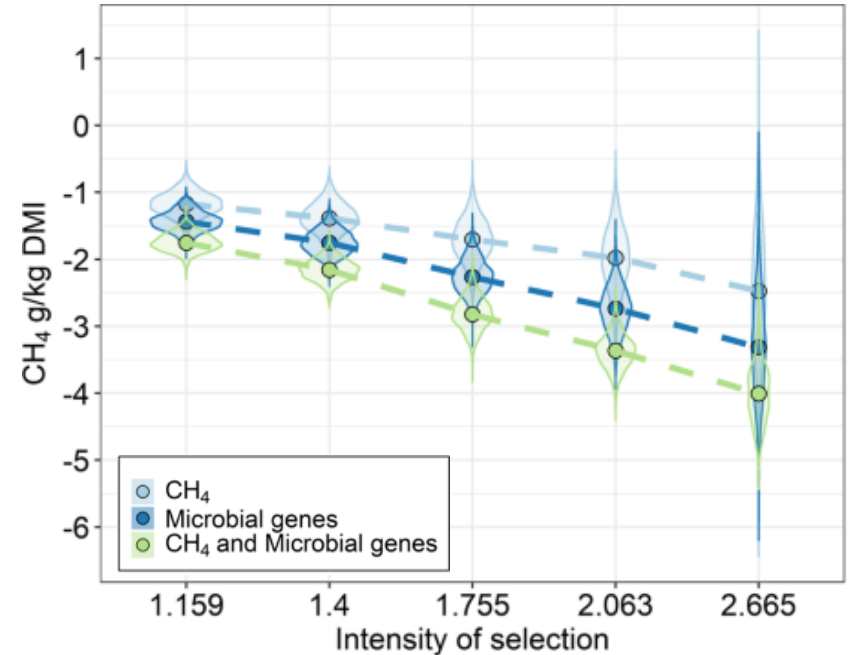


$$P_{\text{henotype}} = G_{\text{enotype}} + M_{\text{icrobiota}} + E_{\text{nvironment}}$$



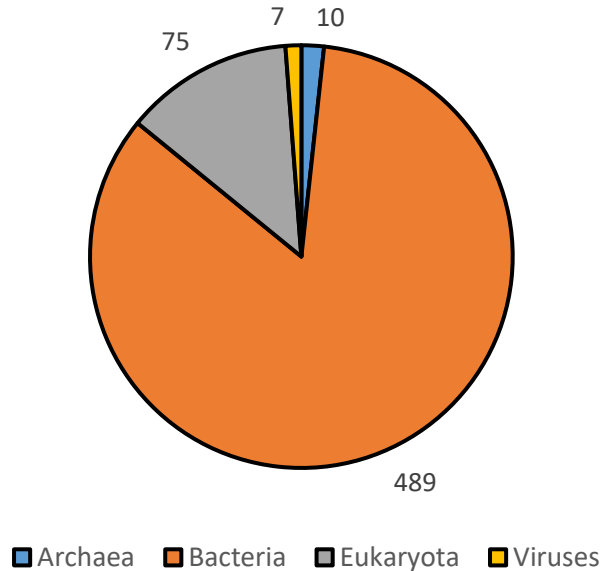
Additional methane reduction

- Selection on combination of methane and microbiome
- Additional reduction in methane of ~4% extra per generation



Microbiome data

Core microbiome composition



PCA of microbiome abundance data

